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Separation and Purification Section:

Summary of Activities
July 1968 to June 1969

U.S. DEPARTMENT OF COMMERCE
NATIONAL BUREAU OF STANDARDS

UNITED STATES DEPARTMENT OF COMMERCE
Maurice H. Stans, Secretary
NATIONAL BUREAU OF STANDARDS • Lewis M. Branscomb, Director

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Separation and Purification Section:

Summary of Activities July 1968 to June 1969

Edited by David H. Freeman
and
Walter L. Zielinski, Jr.

Separation and Purification Section
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FOREWORD

The Analytical Chemistry Division was established as a separate division at the National Bureau of Standards on September 1, 1963, and became part of the Institute for Materials Research in the February 1, 1964, reorganization. It consists at present of nine sections and about 100 technical personnel encompassing some 57 different analytical competences from activation analysis and atomic absorption to vacuum fusion and x-ray spectroscopy. These competences, and in turn the sections which they comprise, are charged with research at the forefront of analysis as well as awareness of the practical sample, be it standard reference material or service analysis. In addition it is their responsibility to inform others of their efforts.

Formal publication in scientific periodicals is a highly important output of our laboratories. In addition, however, it has been our experience that informal, annual summaries of progress describing efforts of the past year can be very valuable in disseminating information about our programs. A word is perhaps in order about the philosophy of these yearly progress reports. In any research program a large amount of information is obtained and techniques developed which never find their way into the literature. This includes the "negative results" which are so disappointing and unspectacular but which can often save others considerable work. Of importance also are the numerous small items which are often explored in a few days and which are not important enough to warrant publication--yet can be of great interest and use to specialists in a given area. Finally there are the experimental techniques and procedures, the designs and modifications of equipment, etc., which often require months to perfect and yet all too often must be covered in only a line or two of a journal article.

Thus our progress reports endeavor to present this information which we have struggled to obtain and which we feel might be of some help to others. Certain areas which it appears will not be treated fully in regular publications are considered in some detail here. Other results which are being written up for publication in the journal literature are covered in a much more abbreviated form.

At the National Bureau of Standards publications such as these fit logically into the category of a Technical Note. We plan to issue these summaries for all of our sections. The following is the third annual report on progress of the Separation and Purification Section.

W. Wayne Meinke, Chief
Analytical Chemistry Division

PREFACE

The year of activity in the pages to follow marks an important transition in the growth of this, the Separation and Purification Section. We now begin our fourth year. The past year involved being on a frontier that was challenging, difficult, and rewarding.

The highlights are the accomplishments of which we are proud. The Section completed its first certification of a Standard Reference Material, an ion exchange microstandard for calcium in the 0.01 to 1 nanogram range. We shared in being among the first groups, our own and one in Czechoslovakia, to detect and measure crosslinking in organic ion exchange networks. We have obtained the best separation to date of the divinylbenzene isomers. We studied copolymer swelling kinetics on beads as small as 10 microns (μm) in diameter.

The frustrations we experienced provide some insight as to problems needing more concerted effort. High pressure liquid chromatography is potentially the most important single analytical tool for organic separations, but we have found the prediction of selectivity to be a subject of confounding difficulty. The provision of purified chemical reagents is in serious need for a suitable container--one free from reactivity, diffusion, brittleness, and impregnated particulates, among other problems. There is a need for development of inorganic microstandard matrices for better sample simulation, this is relevant for geological and possibly for air pollution analysis. There is uncharted territory concerning the chemical applications of homogeneous solid solutions with mixed counterions that are needed as microstandards.

The most difficult challenge was the effort to introduce at NBS a high pressure and high resolution liquid chromatographic discipline, and simultaneously apply it toward the characterization of purity and the separation and measurement of contaminants in clinical Standard Reference Materials. The

chromatography of bilirubin remains perplexing as an unsolved problem of much concern.

The citation of any commercial materials and equipment in this document is occasionally made only to specify procedures which were used, and does not imply recommendation or endorsement by the National Bureau of Standards; nor does it imply the use of such material and equipment as the only suitable sources for the purpose at hand.

Mrs. Janice Hurst, our Section secretary, eased the task of preparing this report and we gratefully acknowledge her patient skills in translating penscript, some of it legible.

David H. Freeman, Chief
Separation and Purification Section

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SEPARATION AND PURIFICATION SECTION: SUMMARY OF ACTIVITIES JULY 1968 to JUNE 1969

Edited by David H. Freeman and
Walter L. Zielinski, Jr.

ABSTRACT

This is the annual progress report on the Separation and Purification Section activities. The major task of developing a certified ion exchange microstandard as a Standard Reference Material is presented in terms of the preparative and characterizational work involved. Fundamental studies of ion exchange substrates includes the application of quantitative analytical infrared spectrophotometry to measure crosslinking in the copolymer network, and to determine the degree of sulfonation. Optical microscopy is applied to the study of swelling kinetics for single copolymer particles. Analytical gas chromatography is applied to the isomers of divinylbenzene. The recently acquired quadrupole mass spectrometer is described. Liquid chromatography at high pressures and with high resolution has been begun recently. The activities of a project dealing exclusively with ultrapure reagents is described including the problems of contamination free storage.

Key words:

Gas chromatography; ion exchange; infrared analysis; liquid chromatography; purification; separation; styrene/divinylbenzene; ultra-pure reagents.

I. ION EXCHANGE MICROSTANDARDS

A. Introduction

A broad range of chemical entities exist in the form of a single element, or combinations of elements, which can be loaded onto ion exchange beads. Following suitable preparation, beads are isolated one at a time. For each given bead, the mass of the element (counterion) in question is determined by measurement of the bead diameter. Bead homogeneity is likewise determined, so that the accuracy of the mass of the element is also known within a well-defined, and rather small, uncertainty. The happiest aspect to emerge from this effort is an important and unique capability for the encapsulation of quantities of matter that are otherwise unweighable. The quantities are sufficiently small to permit novel experiments and calibration of chemical measurement systems, especially those designed for handling very small samples.

Several adaptations of this technique have evolved by acceptance into other laboratories. Thus, the preparation of micro sources of radioactivity has been carried out by Paul B. Hahn at the Northeastern Radiological Health Laboratory (HEW-PHS). Dr. Lynus Barnes (Analytical Mass Spectrometry Section, NBS) has developed a method of standard addition that permits quantitative mass spectrometric analysis at the nanogram level. The combined co-calibration of the electron microprobe for magnification and for elemental ratio (counterion to sulfur) has been worked out by Robert Myklebust and Dr. Kurt Heinrich (Spectrochemical Analysis Section, NBS). The results of all of these studies will be made public at the forthcoming Eastern Analytical Symposium (New York, New York, November 1969). The observation that these and other disciplines are engaged in extending the feasibility of using these standards, coupled with the continuing improvement of detector sensitivity limits, indicates their growing potential to a broad spectrum of chemical microanalysis.

In the previous annual report [1], we outlined the general capability of ion exchange microstandards. A full description of their preparation and testing is planned for publication in the near future so that a duplication of this information will not be given here. The following section will provide coverage of the highlights of the past year's activities in this area. A separate project involves the development of larger particles that are to be used in the nanogram to microgram mass range, and these efforts are described in Section II of this report.

B. Preparation

Ion exchangers have the obvious capability for exchanging ions. The preparation of an ion exchange standard depends upon two important requirements. The placement of the ions must be done uniformly. Once placed, the ion composition must be locked against accidental change. The former depends upon access to an intrinsically homogeneous ion exchange network. These conditions are not obtained without special care in synthesis, and the proof of homogeneity involves an experimental effort that we have approached comprehensively.

The preparative methods are monitored both for completion of ion placement and for possible contamination. In column operations, we may start with a sodium form exchanger, since this can be monitored using the sodium selective electrode, shown in Figure 1. The prepared exchanger is tested for traces of residual sodium content using flame photometry.

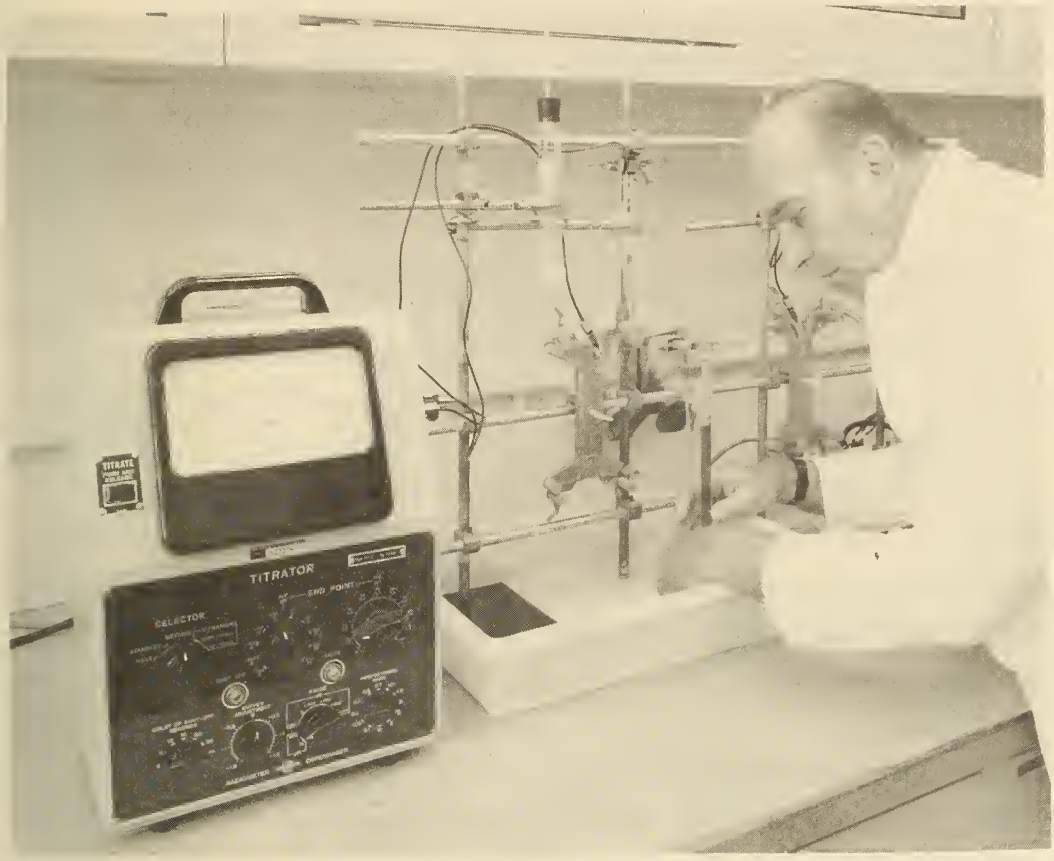


Figure 1. Sodium Ion Selective Electrode Used for Monitoring Exchanger Effluent Stream.

C. Casting

The exchanger in its prepared form is washed with solvents that deswell the network and the material is dried. The dry exchanger is then immersed in a pure, inert and nonswelling liquid, such as cyclohexane. The bead clusters are dispersed ultrasonically and a dilute suspension is cast onto glass slides. Pretreatment of the glass microscope slides involves exhaustive leaching to extract potentially exchangeable impurities from the glass surface. While glass is not the best possible choice of container, as we have reported in a recent summary [2], it does offer the advantage of providing just enough electrostatic attraction to hold small beads in place once they are carried by the hydrocarbon liquid onto the surface. This was tested in some detail since we had to

show that such beads, once cast onto a surface and then sold as Standard Reference Materials, would remain in place. In routine tests, the beads adhered to the glass despite repeated drop tests from an altitude of three meters above the floor.

D. Stripping

To function as a microstandard, the adsorbed element must be released from the matrix of the ion exchange bead. If the matrix is not to be destroyed *per se*, the problem is one of eluting (stripping) a micro ion exchange system; understanding that the procedure would be the same as for a macro system. In the case of nanogram quantities on micron-size beads either too large a volume of extracting agent or too lengthy an extraction process would hamper the utility of a microstandard and the potential for loss would likewise become extreme.

For simple monovalent ions, stripping presents no difficulty; water alone is sufficient, so much so that high room humidity can cause loss through leaching. With multivalent ions stripping becomes more difficult; the ions are adsorbed so strongly that a small amount of water cannot effect complete removal.

Other possible methods of elution include washing with complexing agents, or oxidation-reduction of the ion while it is adsorbed on the resin.

The first microstandard where stripping became a problem was chromium, adsorbed as Cr (III). Nelson, *et al*. [3] have indicated that either concentrated or dilute hydrochloric acid could effect stripping. Qualitative tests showed however, that though results could be achieved using concentrated acid, the process was a lengthy one with complete extraction not assured. EDTA showed the same behavior.

More satisfactory results were obtained by oxidizing the adsorbed chromium with alkaline hydrogen peroxide. In a matter of seconds the yellow color of the chromate ion could

be seen in solution. Preliminary quantitative measurements indicate complete removal of the chromium from the resin.

E. Analysis

Activation analysis provided a significant test for the five kilogram sample of 5-25 μm beads that were used in the initial effort in the development of ion exchange micro-standards. The results are shown in Figure 2. The experimental

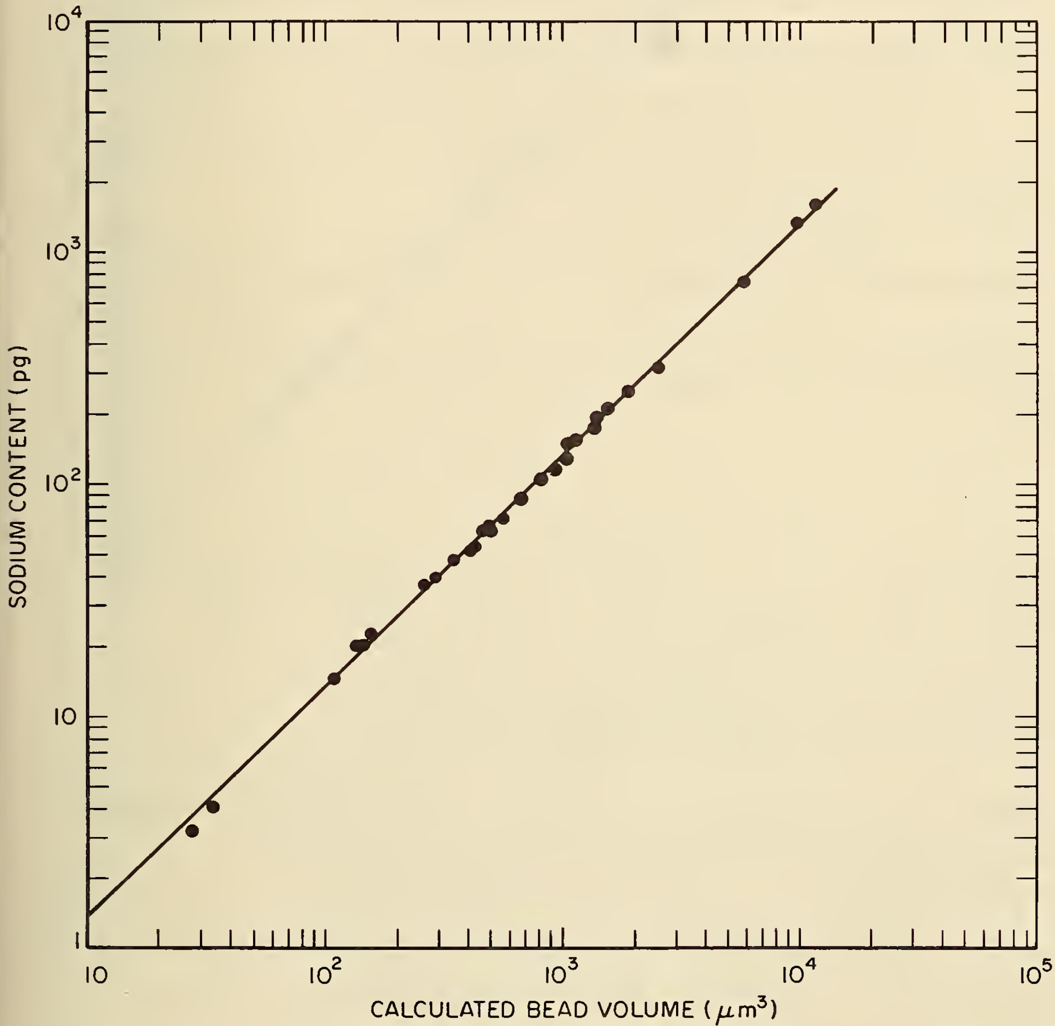


Figure 2. Analysis of Exchanger Loadings by Activation Analysis.

particulars will be discussed in detail elsewhere, but it has become obvious that the measured sodium content revealed a direct uniform variation of counterion content with the microscopically measured bead volume. The variations do not extend appreciably beyond the anticipated measurement error. It is significant to note that these results involve 10^{-4}-fold smaller quantities of matter than reported previously when this type of test was first announced.

The electron microprobe analysis of the Ca-form ion exchange beads showed a stable Ca/S ratio for the respective K_α x-rays. The microprobe results as reported [4], imply suitability of the beads as correspondence standards between signal ratio and absolute elemental ratio.

The effect of particle size upon microprobe x-ray signal was quite informative. The result for Ca is shown logarithmically in Figure 3. The results for S were identical in trend, as expected. The slope of two indicates the relationship

$$(Ca) \text{ or } (S) \propto d^2 \quad (1)$$

where d is particle diameter. This is the same as the predicted behavior for an electron (or x-ray) opaque bead in which penetration toward the bead core is slight. In terms of the need to measure possible bead heterogeneity however, shallow penetration is desirable for the following reason. Given the independent network heterogeneity determination provided by activation analysis, the major possible source of heterogeneity should be traceable to surface loss by ion exchange. Electron microprobe analysis therefore, offers a significant contribution by narrowing its search to this important region of the bead.

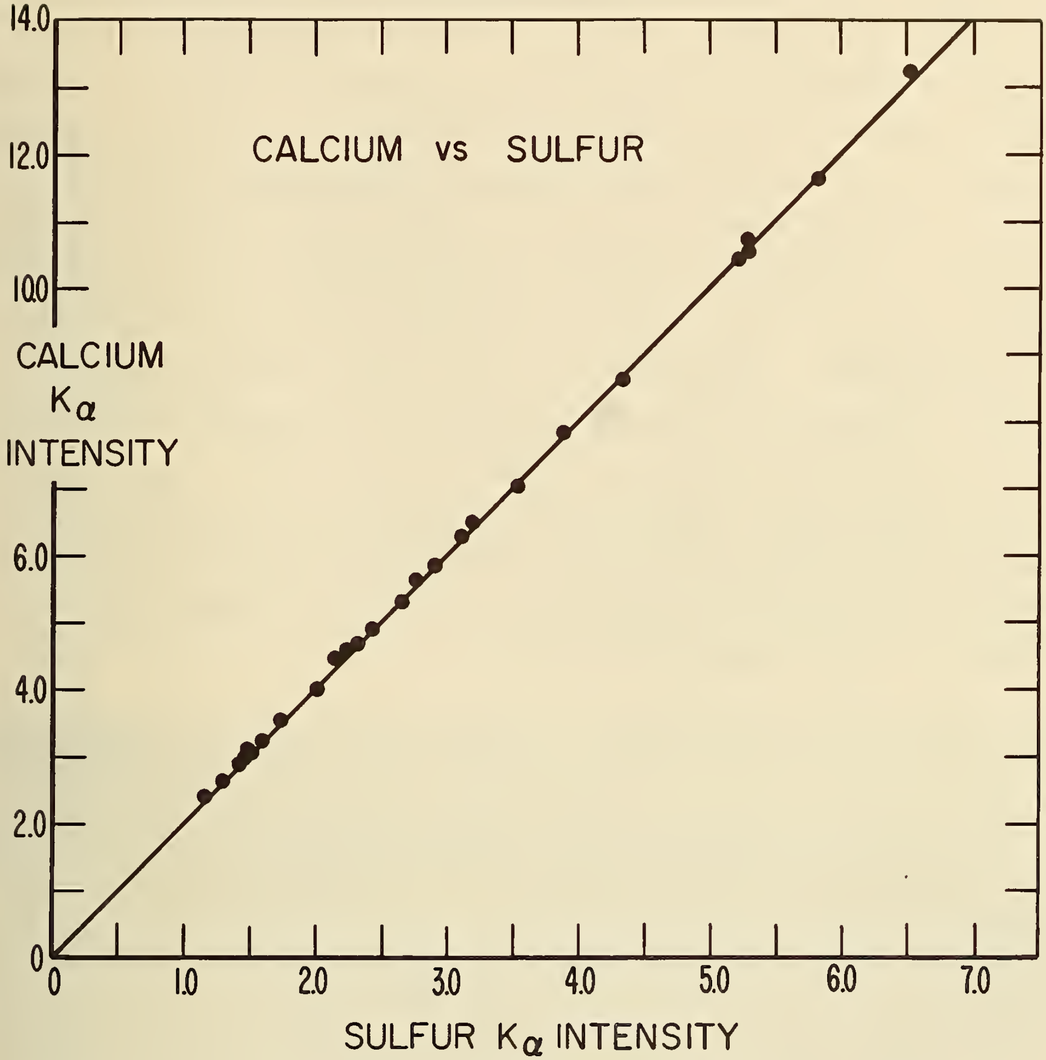


Figure 3. Effect of Bead Size Upon Calcium Analysis by the X-Ray Microprobe.

F. Other Measurements

It should be noted that other contributions to this project have been carried out and will be reported elsewhere. The determinations of (a) ion exchange capacity, (b) matrix element measurements and (c) the anhydrous exchanger density have been performed with the cooperation of R. A. Paulson (Microchemical Analysis Section, NBS). Measurements of counterions using polarographic [5] and flame photometric methods have also been carried out [6].

One aspect of this interlaboratory work is the common feature that the equivalent weight of the exchanger network (excluding counterions) should be independent of the counterion form i. The exchanger equivalent weight E (g/eq) is the sum of the network (E_N) contribution and counterion ($E_I = \Sigma y_i E_i$) contribution, where y_i is the equivalent fraction and E_i is the equivalent weight of the particular counterion. Note that for $y_i = 1$, $E_i = E_I$. In Table I is a listing of various independent values of E_N. Any one of these determinations is considered accurate at the 0.5 percent level, and the differences are in all probability, real. The variations should be a composite indication of the measurement error in addition to the real physical or chemical difference. Sample heterogeneity, to be sure, tends to be ruled out by single particle assays.

Table I. Network Equivalent Weight (E_N) Measurements

Counterion	% by Wt.	Eq. Wt. (Gross)	E_N †
Na^+	10.36	222	199
Na^+	10.5	219	196
Ca^{++}	8.91	225	205
Ca^{++}	9.10	220	200
Pb^{++}	34.6	299	196
Cu^{++}	14.0	227	195
Cu^{++}	13.3	239	206
H^+	0.519	195	193

† Note: For a single counterion $E_N = E_I \left(\frac{1 - f_I}{f_I}\right)$ where f is wt. fraction of that counterion.

G. Measurement, Transfer and Packaging

From independent efforts, we know that liquid water contains a small amount of electrolyte. This can result from water hydrolysis, or from reactions following the adsorption of atmospheric gases such as carbon dioxide. For these reasons, a microscopic amount of water condensation is sufficient to cause ion exchange, and that of course would tend to ruin a given microstandard. To minimize this possibility, we have maintained a practice of restricting the humidity to less than 50 percent of relative humidity at temperatures near 23° C.

The elimination of atmospheric dust is an equally desirable condition. For that reason, we have carried out our operations in a specially constructed clean room facility (Figure 4) which is used for casting and packaging operations

Figure 4. Clean Room Facility for Casting and Packaging of Microstandards.

for the issuance of the ion exchange beads as Standard Reference Materials. In Figure 5 is a photograph of a less elegant, but similar facility in which the room has been constructed out of polyethylene. Inside the room is a laminar flow clean bench. The air in the room is principally recirculated by an air dehumidifier/air conditioner.

H. Certification

The first microstandard to be completed is that for calcium in the region of 0.01 to 1 nanogram. This exciting and uniquely minute encapsulation of the alkaline earth cation is prepared as castings upon the microscope slides. A minimum of one hundred isolated beads are adherent to the glass surface. The beads are readily picked up on a clean fine-tipped probe.

It is of interest to include the first certificate which has been issued for these individual ion exchange bead microstandards. The certificate is displayed in Figure 6.

Fig. 5. Polyethylene Clean Room.

U. S. Department of Commerce
Maurice H. Stans
Secretary

National Bureau of Standards
A. V. Astin, Director

Certificate

Standard Reference Material 1800
Microstandard Ion-Exchange Beads
Calcium Loaded

(10^{-11} to 10^{-9} grams)

This Standard Reference Material is intended as a source of minute, known quantities of calcium. The calcium is attached to ion-exchange beads that are 5 to 25 microns (μm) in diameter. The matrix is composed of a homogeneously sulfonated network of a cross-linked copolymer of styrene with 8 mole percent *m*-divinylbenzene. The calcium is electrostatically attached to the sulfonate groups. The calcium ions can be displaced by addition of an aqueous electrolyte solution.

Any single bead contains an amount, m, of calcium, sulfur, carbon, hydrogen, oxygen (and some water) according to the equation: $m_{Ca} = \rho_{Ca}V$, where $V = \pi d^3/6$ is the volume of the bead, and m_{Ca} and ρ_{Ca} are the mass and partial density, respectively, for calcium. The partial density of calcium in a typical dry bead is certified as: $\rho^{\circ}_{Ca} = 0.135 \pm 0.010$ g/cm^3. The superscript ($^{\circ}$) is used to denote reference to a dry resin bead. The value of ρ° and its error (standard deviation) is computed from neutron activation analysis and from electron microprobe analysis of forty individual beads. The microprobe measurements also agree with the expected constancy in the calcium to sulfur ratio.

Analysis of gross quantities of this material gives the partial densities of the subscripted matrix elements $\rho^{\circ}_{S} = 0.21_5$, $\rho^{\circ}_{C} = 0.71$, $\rho^{\circ}_{H} = 0.06$. The bulk density of the dry material ranges from 1.49 (minimum) to 1.50 (maximum) g/cm^3.

The preceding measurements refer to the material after vacuum drying at 120 °C for 16 hours. If exposed for two weeks or more to ordinary atmospheres with relative humidities in the range 20 to 40 percent, the partial densities will be reduced according to the amount of sorbed water. Then, the partial density of each of the elements of the bead must be adjusted by multiplying by the factor 0.87. In the case of calcium, the corrected partial density is: $\rho_{Ca}(20\text{–}40\%\ \mathrm{RH}) = 0.117 \pm 0.009$ g/cm^3.

PROVISIONAL

Washington, D. C. 20234
March 17, 1969

W. Wayne Meinke, Chief
Office of Standard Reference Materials

CAUTION: Inadvertent contamination by water, *i.e.*, condensation, is sufficient to cause the loss of calcium by ion exchange hydrolysis. It is therefore essential that this microstandard be kept at less than 50 percent relative humidity.

(over)

Figure 6. First Microstandard Certificate: Calcium.

Measurements leading to the certification of this Standard Reference Material were carried out by D. A. Becker, L. A. Currie, H. D. Dixon, R. A. Durst, K. F. J. Heinrich, E. C. Kuehner, R. L. Myklebust, R. A. Paulson, T. C. Rains, and G. Schmuckler of the Analytical Chemistry Division.

The overall direction and coordination of the preparation and technical measurements leading to the certification of this material were performed under the chairmanship of D. H. Freeman.

The technical and support aspects involved in the preparation, certification and issuance of this Standard Reference Material were coordinated through the Office of Standard Reference Materials by T. W. Mears.

USE OF MICROSTANDARD

The present microstandard was prepared by casting a dispersion of dry, calcium-form, ion-exchange beads onto a clean glass slide. The beads are naturally attracted to the glass surface. The same attraction occurs when the beads are touched gently with a probe, and this permits them to be moved from the slide to another place.

DIAMETER MEASUREMENT: Under microscopic examination it will be observed that the majority of the beads in this standard are spherical. The nonspherical beads are readily identified and rejected on the basis of their appearance. Bead diameter measurements are then practicable to an estimated total uncertainty of $\pm$ 0.1 micron (μm). Such measurements are not difficult, but they do require proper microscopic measuring apparatus and illumination technique (such as Köhler illumination with the use of an interference filter at $\approx$560-nm). These procedures are described in the literature (D. H. Freeman, "Advances in Ion Exchange," Volume 1, Marcel Dekker, Inc., New York, 1966, chapter 5).

STORAGE: The microstandard beads must be protected against exposure to excessively humid atmosphere and, for practical purposes, it should be assumed that the standard is destroyed if exposed to humidities exceeding 50 percent relative humidity. Storage over a mild desiccant is recommended. Alternatively, the beads may be vacuum dried at 120 °C for approximately 16 hr in order to duplicate the dry state to which the composition measurements refer. After drying, the beads can be kept in the dry state by storing them over fresh phosphorous pentoxide, or they can be immersed in a dry hydrocarbon fluid. There is some loss in accuracy (but a gain in convenience) by working in atmospheres of controlled humidity. For example, isopiestic equilibration will occur after approximately two to four weeks of storage over an aqueous calcium chloride solution in the range from 0.2 to 0.4 water activity (approximately 7.2 moles $CaCl_2$ per kg of H_2O for 30% relative humidity).

These standards can be used in a variety of ways. A single bead can be treated with electrolyte to release the ion-bound calcium. Or, a bead can be observed destructively as in the laser microprobe where the sample is vaporized and then analyzed by emission spectroscopy. Single beads can be viewed by an instrument such as the electron microprobe which is capable of identifying and measuring relative amounts of the calcium or sulfur atoms contained by the beads or by comparative samples.

II. ION EXCHANGE SUBSTRATES

A. Introduction

The microstandards issued recently [1,7] are based on the availability of stable, reproducible and accurately characterized poly[styrene/divinylbenzene] beads. These copolymer beads serve as precursors for a wide variety of possible ion exchange derivatives. In the case of micro-standards, we have employed sulfonated beads. The properties of the gel network are dependent upon the careful control of the synthetic conditions, including monomer composition. Specifically, both monomers should be pure, and their concentrations should be accurately known. Other conditions, such as the bead size-range and the polymerization rates are equally important. The subsequent reaction on the network (sulfonation) likewise requires careful control. It has been shown that such conditions can be achieved [8].

The researcher faced with using ordinary ion exchange materials may show small or large variations in the properties of different batches of supposedly identical networks. To illustrate, the ion-exchange capacity will vary from batch to batch, and it seldom approaches its ideal value. In this area in particular, simple definitive tests are lacking to evaluate the substrate properties.

With such considerations in mind, three areas of investigation were undertaken; viz. the synthesis, the characterization, and the structure of sulfonated poly[styrene/divinylbenzene] and its parent copolymers. These studies are more fully described below.

B. Preparation

1. Synthesis of Copolymer Beads

The first problem in this area lies in obtaining pure monomers. Styrene presents no difficulty, but either meta- or para-divinylbenzene (DVB) is difficult to purify [9].

Purification procedures, analytical methods and procurement channels have been determined [10]. One experienced manufacturing firm in the copolymer synthesis field (Ionac Chemical Co., New Brunswick, New Jersey), agreed to prepare beads to meet our specifications* using standard methods [11] with purified divinylbenzenes. Accordingly, a stock of poly [styrene/DVB] copolymers was synthesized for analytical and structural studies. The crosslinking was varied according to isomer (meta- or para-DVB) type and concentration, with all other synthetic conditions held constant. The copolymers are listed in Table II. Among those listed is 8 mole-percent meta-divinylbenzene crosslinked copolymer (poly[styrene/0.08 m-DVB]) which on sulfonation is the substrate for the ion exchange microstandards.

In order to compare the pure copolymers with commercial samples, a synthetic investigation is in progress with Dr. A. H. Greer (Ionac Chemical Co.) to determine which of several experimental variables relate to the swelling behavior which has been observed. This closely coordinated synthetic approach is expected to provide structural insight for the evaluation of fundamental ion-exchange properties. Similar approaches have been used by other workers [8,12].

2. Sulfonation of Copolymer Beads

Careful control of copolymer sulfonation is a necessary prerequisite in the development of reliable cation exchange networks. One type of experiment using pre-sized beads suggests that sulfonation may be a two-step process as indicated in

*These specifications require the use of pure DVB (supplied by us) within ± 2 percent concentration accuracy; styrene of the same concentration accuracy; specified particle size range; and the production of optically clear, strain-free, spherical beads.

Table II

Copolymer Beads with Known DVB Crosslinking

Ident. Number	Comments	Mole % DVB Meta	Mole % DVB Para
I-19	Poly(Styrene/DVB)		1.0
I-20	Poly(Styrene/DVB)		2.0
I-21	Poly(Styrene/DVB)		4.0
I-22	Poly(Styrene/DVB)		8.0
I-23	Poly(Styrene/DVB)		16.0
I-25	Poly(Styrene/DVB)	1.5	
I-110	Poly(Styrene/DVB)	2.0	
I-111	Poly(Styrene/DVB)	4.0	
I-112	Poly(Styrene/DVB)	8.0	
I-24	Poly(Styrene/DVB)	16.0	
I-11	Microfine Poly(Styrene/DVB)	8.0	
I-126	Poly(Vinyl toluene/DVB)	8.0	
I-127	Solvent Modified 10% Xylene, Poly(Styrene/DVB)	8.0	
I-128	Solvent Modified 50% Xylene, Poly(Styrene/DVB)	8.0	
I-129	Poly(Styrene/DVB)	5.6	2.4

Figure 7. These data imply that the final few percent of sulfonation occur in weeks as compared to hours for major sulfonation of the beads. Other results have demonstrated that oleum ($H_2SO_4 + SO_3$) under mild conditions is a rapid sulfonating agent when the proper solvent system is used [13].

For certain purposes, surface sulfonation is desirable. Figure 8 indicates the result obtained in such an instance.

High purity reagents and careful control of concentrations are most essential. A technique for preparing pure (100 percent) sulfuric acid is illustrated in Figure 9.

A single literature reference (Pepper *et al.* [14]) has indicated that ideal sulfonation (one sulfonic acid group

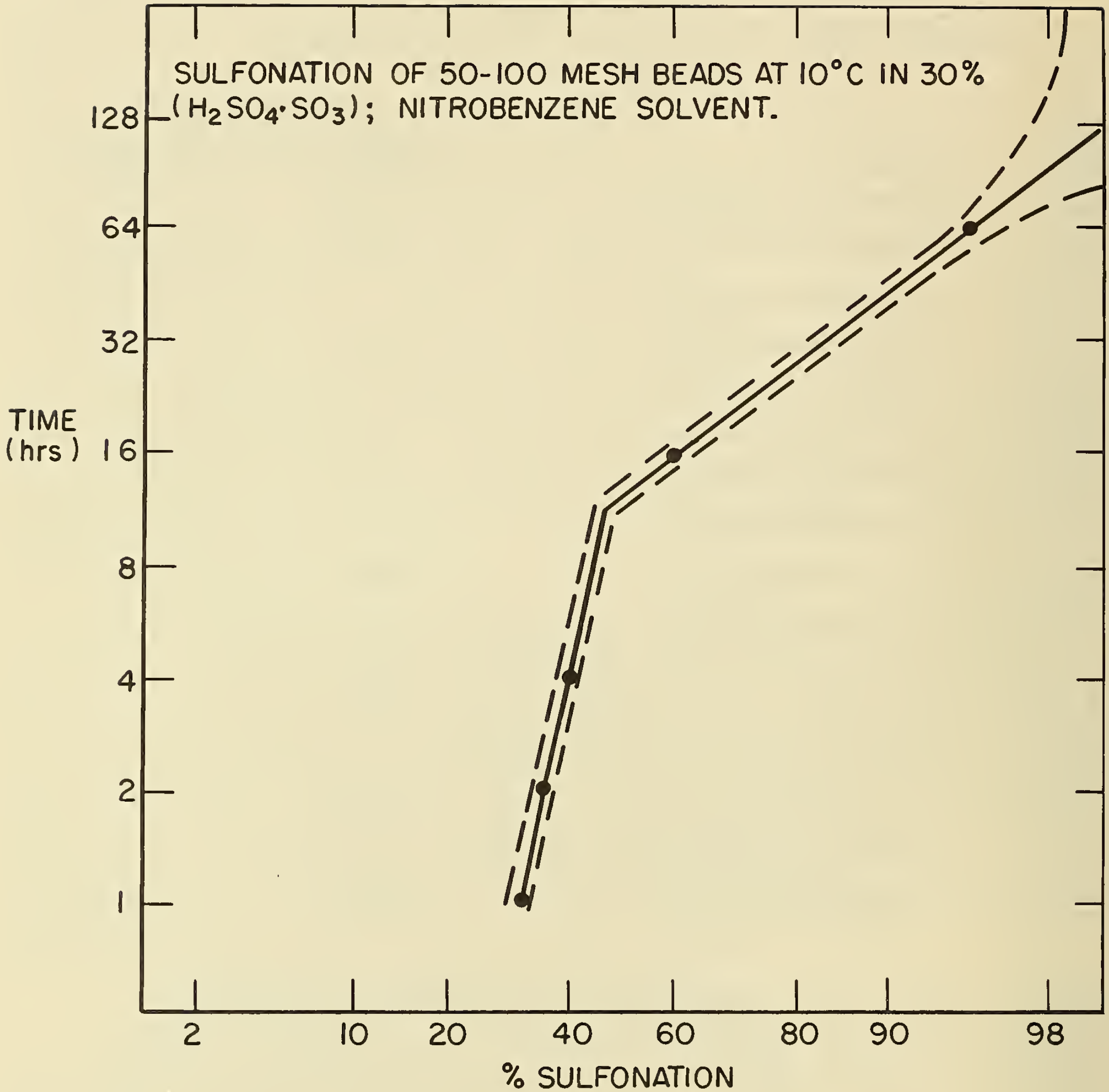


Figure 7. Copolymer Sulfonation of Pre-Sized Beads.

Figure 8. Surface Sulfonation of Copolymer Beads.

Figure 9. Preparation of 100% Pure Sulfuric Acid by Slow Crystallization at 9° C.

per aromatic residue) of the copolymer is attainable. Other workers claim this achievement is not possible [15]. Employing the same catalyst (silver ion) as Pepper, we have found that Pepper's conclusion is valid, even though our sulfonation system was not H_2SO_4, but oleum.

This result permits a greater accuracy in preparing beads of high capacity and low variability; a factor of great importance in the preparation of beads for microstandards in the micro- and nano-gram range.

C. Characterization

1. Infrared Spectrophotometry of Copolymers

Beads prepared with pure DVB (Table II) were used to evaluate qualitatively and semi-quantitatively the crosslinking with respect to both DVB isomer type and mole percent from IR spectral analysis. Polystyrene* was used as a reference standard.

The infrared spectra were obtained from a grating infrared spectrophotometer (Figure 10). The instrument was calibrated to within an error tolerance of 0.5 cm^{-1} using ammonia vapor and indene liquid for the region 910-790 cm^{-1}. The copolymer beads were pretreated by soxhlet extraction for 16 hours with benzene and then vacuum dried. The beads were pulverized at liquid nitrogen temperature in steel capsule containing a steel ball. The procedure of powdering the copolymer was carried out with an electric vibrator (Wig-L-Bug, Crescent Dental Company, Chicago, Illinois) for two minutes. Four mg of the powdered copolymer

*N.B.S. Standard Reference Material 705 (Narrow Range Polystyrene).

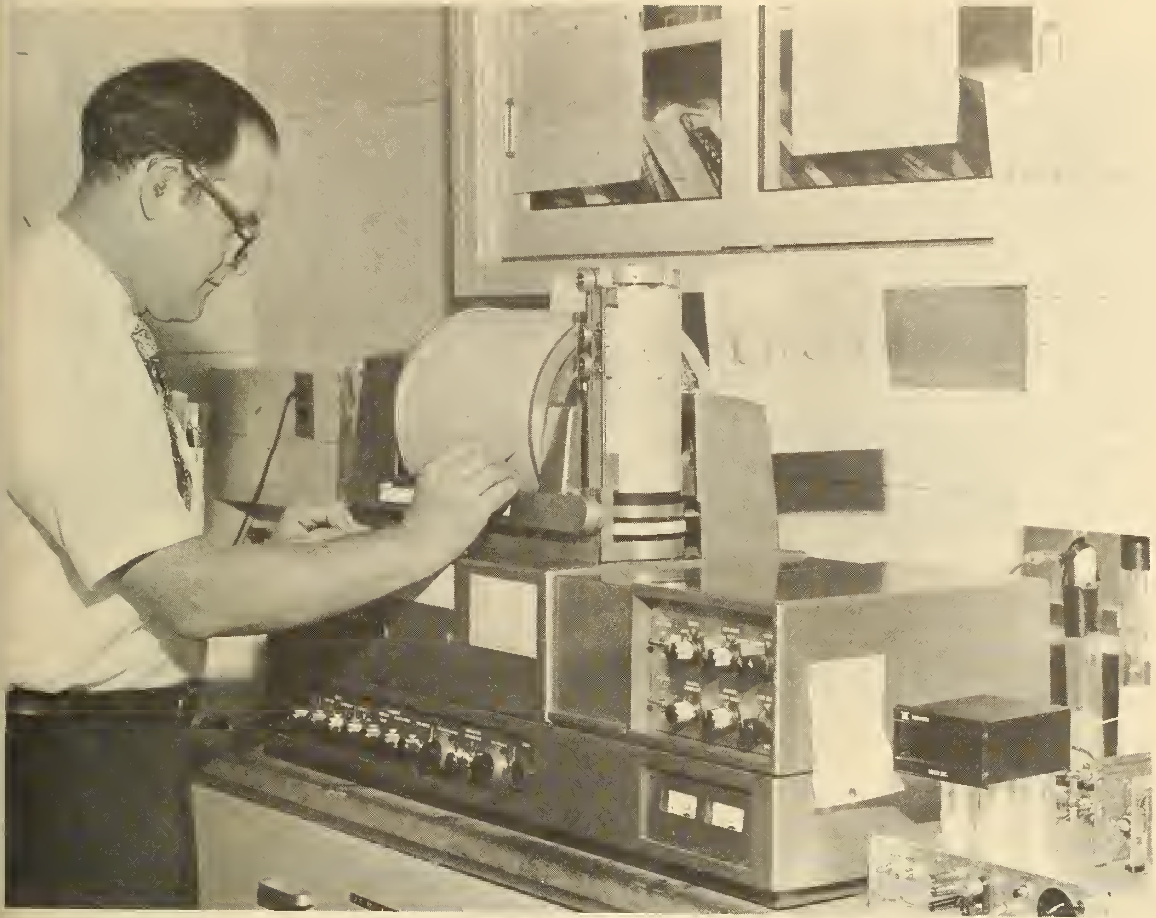


Figure 10. Grating Infrared Spectrophotometer.

were then mixed with 300 mg of KBr and the mixture was again vibrated for one minute at room temperature. The KBr wafer was formed in a 13 mm die using 15 tons of pressure.

Although the full frequency range of 4000 to 400 cm^{-1} was examined (Figures 11-14), attention was focused on a band at 797 cm^{-1} in poly(styrene m-DVB) copolymers, and on the lower frequency band of a doublet near 830 cm^{-1} in poly (styrene p-DVB) copolymers (Figure 15). Both vibrations are due to out-of-plane aryl hydrogen bending, their intensities suggesting the amount of crosslinked meta- and para-DVB present, respectively. Reference to Figure 14 indicates that both types of bridging are present in technical copolymers.

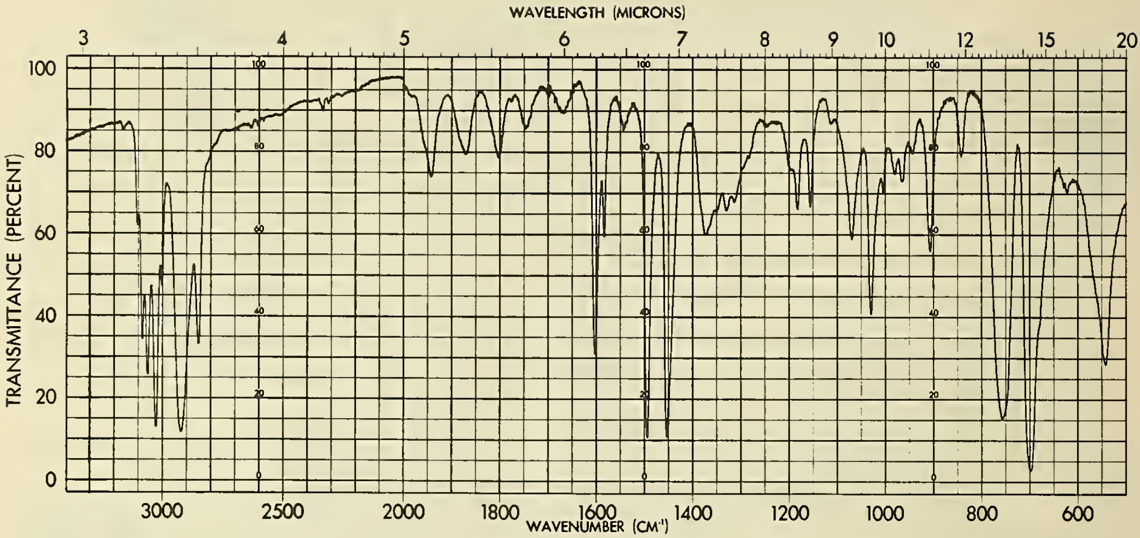


Figure 11. Infrared Spectrum of Polystyrene.

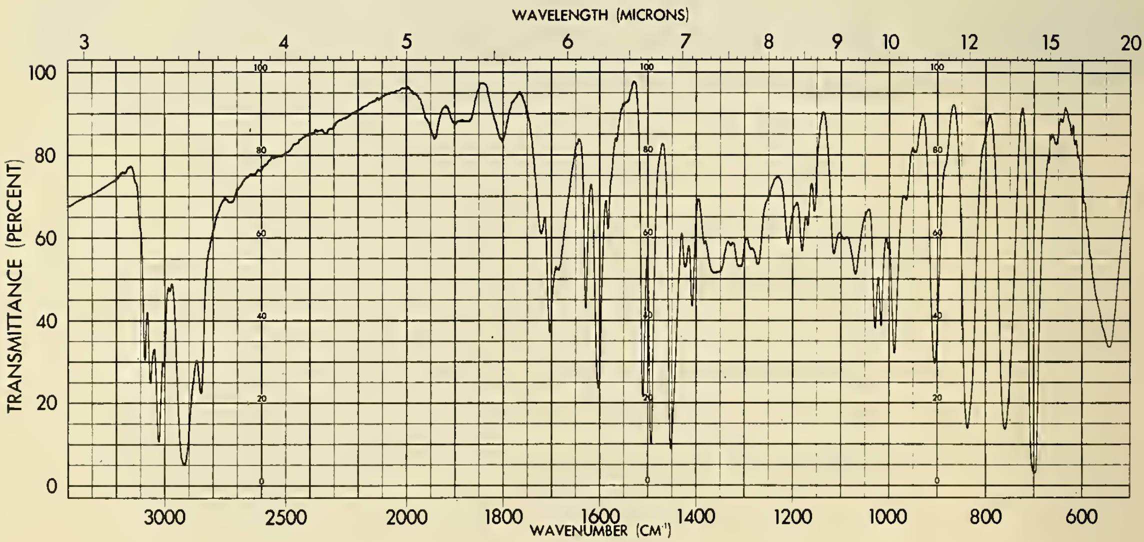


Figure 12. Infrared Spectrum of Poly(styrene/ 0.16 p-DVB).

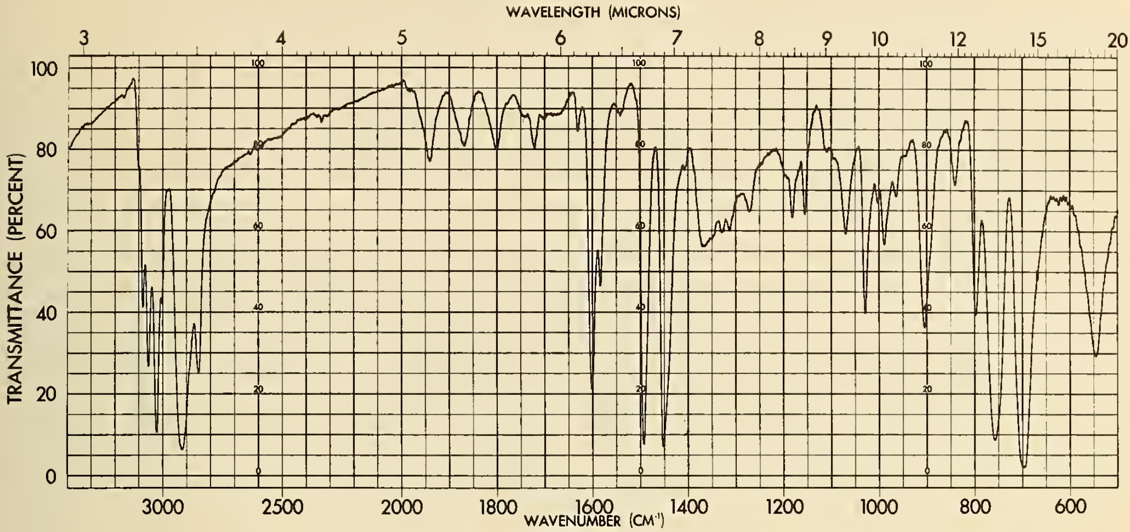


Figure 13. Infrared Spectrum of Poly(styrene/ 0.16 m-DVB).

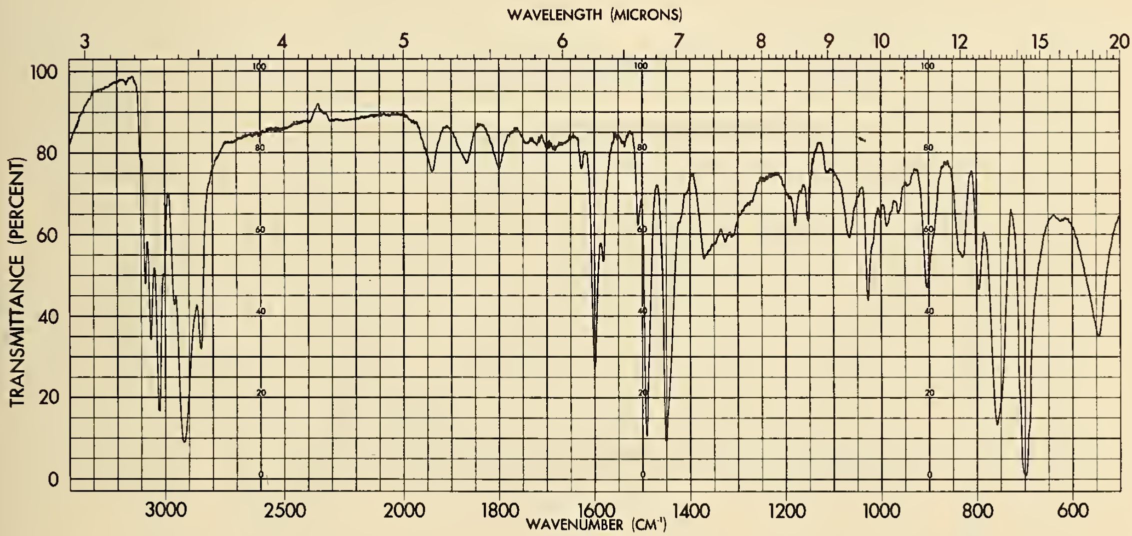


Figure 14. Infrared Spectrum of Poly(styrene/ 0.16 technical DVB).

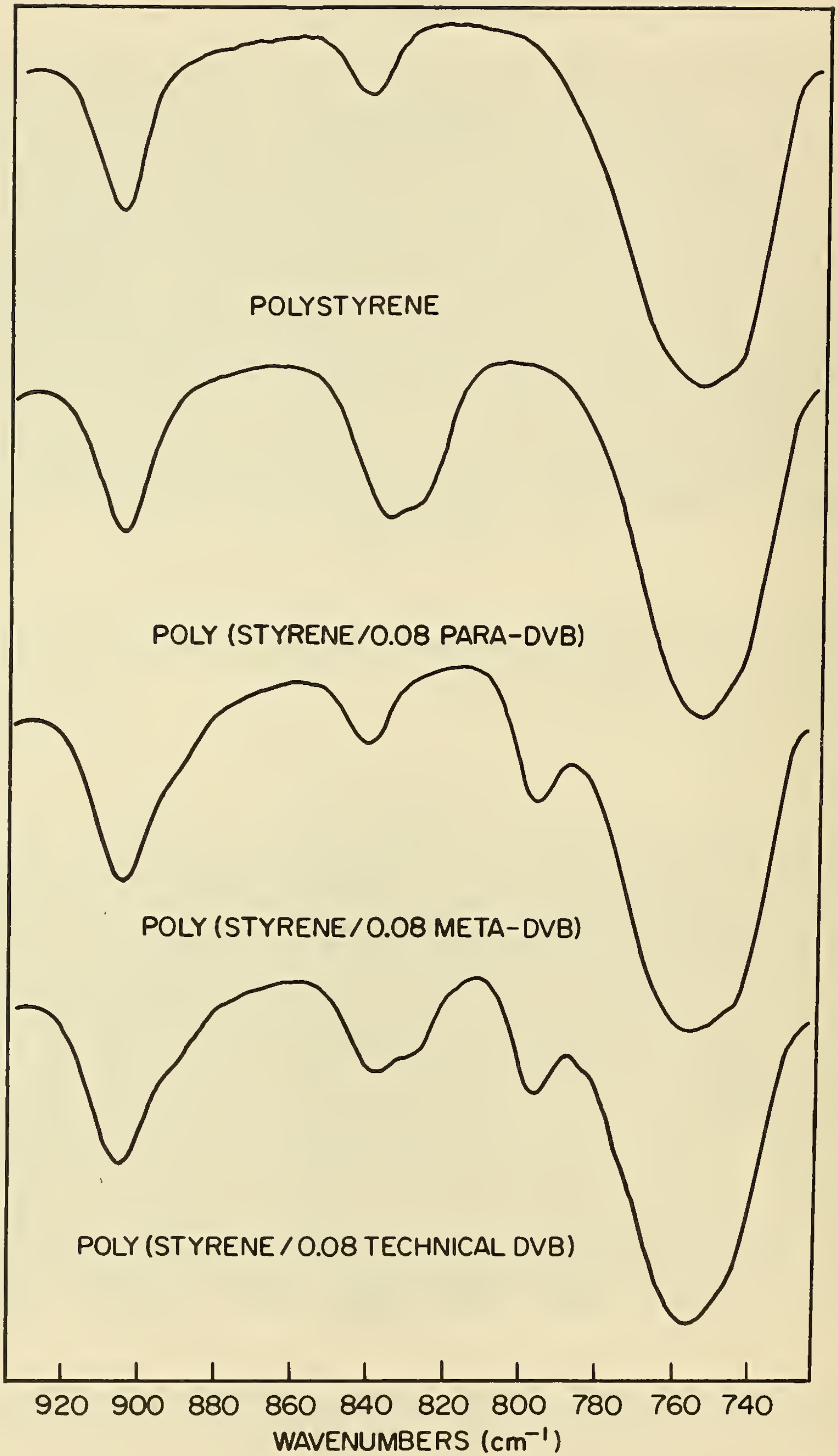


Figure 15. High Resolution Spectra of Polystyrene and Poly(styrene/DVB) Copolymers.

Indeed, the technical DVB often employed in copolymer synthesis usually contains less than 50 percent meta- and para-DVB (in an approximate 2.5:1 ratio); where gas chromatographic analysis reveals the presence of meta- and para-ethylvinylbenzene, at least three other vinyl aromatic compounds, and two dozen other impurities [16,17].

The relationship of infrared absorbance intensity relative to an increasing concentration of meta-DVB in authentic poly(styrene m-DVB) copolymers is shown in Figure 16. No such simple relationship exists for para-DVB copolymers however, owing to the overlapping of the aryl hydrogen out-of-plane band of para-DVB bridges with a band of a similar vibrational mode due to the polystyrene chain. Computer analysis of the digitized doublet indicated resolved bands at 838.5 cm^{-1} for the polystyrene band, and at 826.2 cm^{-1} for the para-DVB band. The correlation study of para-DVB

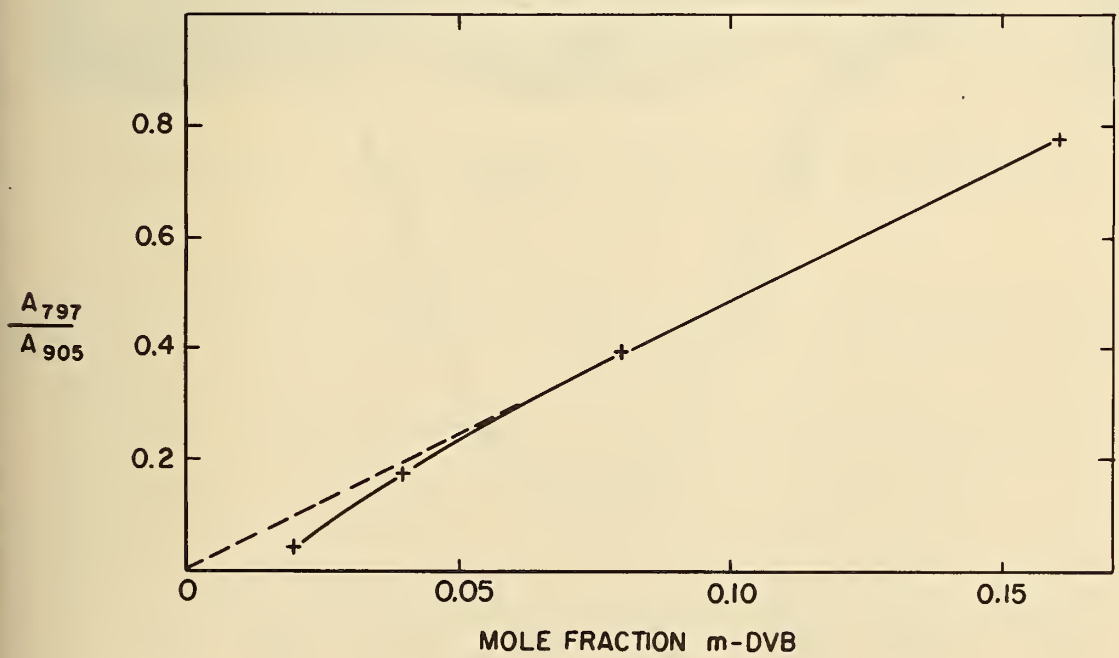


Figure 16. Absorbance Ratio of m-DVB Band Relation to Mole Fraction of m-DVB (Base Line Method).

content in poly(styrene p-DVB) copolymers with infrared absorbance intensity, will continue. The resolved doublet curve is given in the Cal Comp plot of Figure 17. A device for automatic digitization of infrared spectra is being designed in our Section (Figure 18).

2. Infrared Spectrophotometry of Sulfonated Copolymers

Using techniques similar to those described above, it was found possible to quantitate the 1408 cm^{-1} peak of the spectra in partially sulfonated samples [18,19]. The resulting curve of percent sulfonation versus absorption (Figure 19) shows a non-zero intercept, as also noted for m-DVB copolymers above (Figure 16).

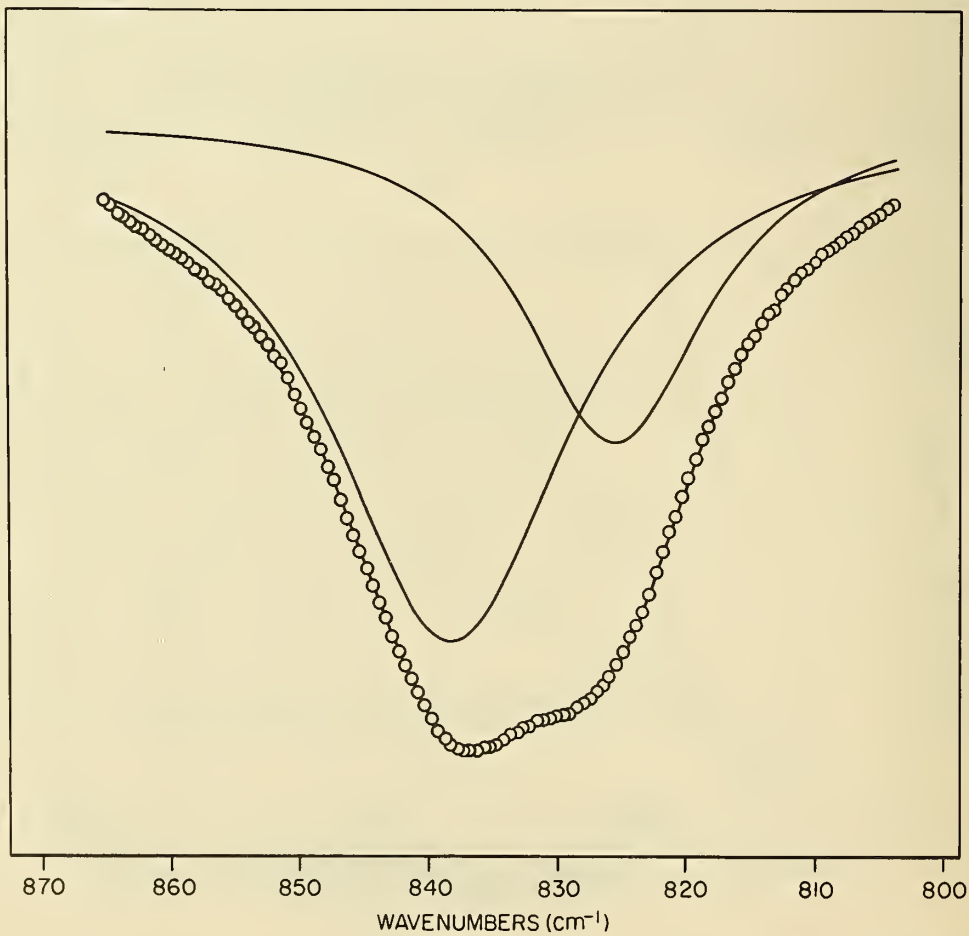


Figure 17. Cal Comp Plot of Digitized Poly(styrene/0.08 p-DVB) Unresolved and Computer Resolved Doublet.

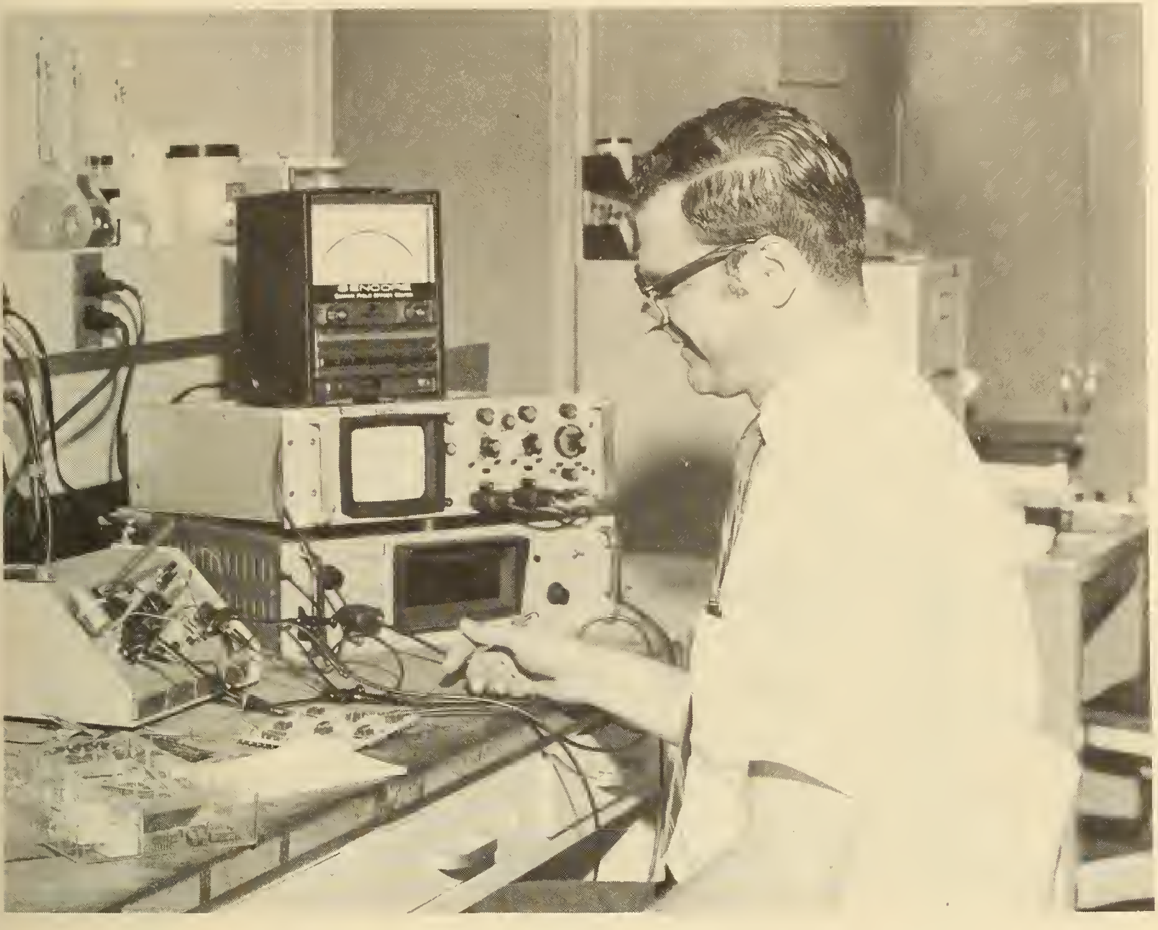
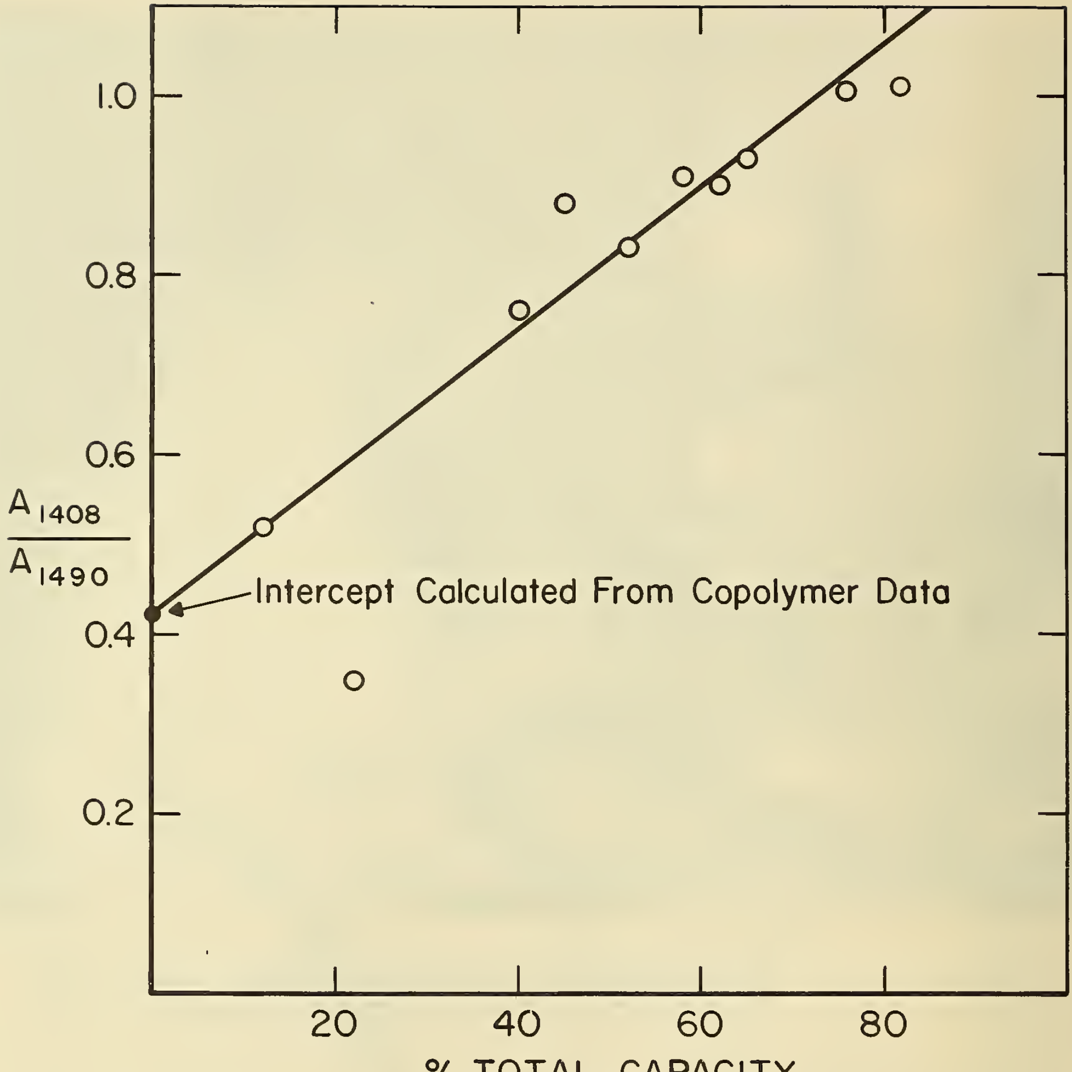


Figure 18. Preliminary Testing of Design Circuitry for Automatic Digitizing Device.

3. Swelling Studies

It is generally known that certain physical properties of copolymer beads are relevant to the network structure, although details are less than fully understood. With this in mind, a homogeneity test on the copolymer that is simple and precise is attractive. Microscopic measurement of the swelling of individual copolymer beads was found to give both kinetic and thermodynamic data. These data are consistent in terms of the known DVB content and the swelling ratio of swollen to dry volume, as shown in Figure 20.

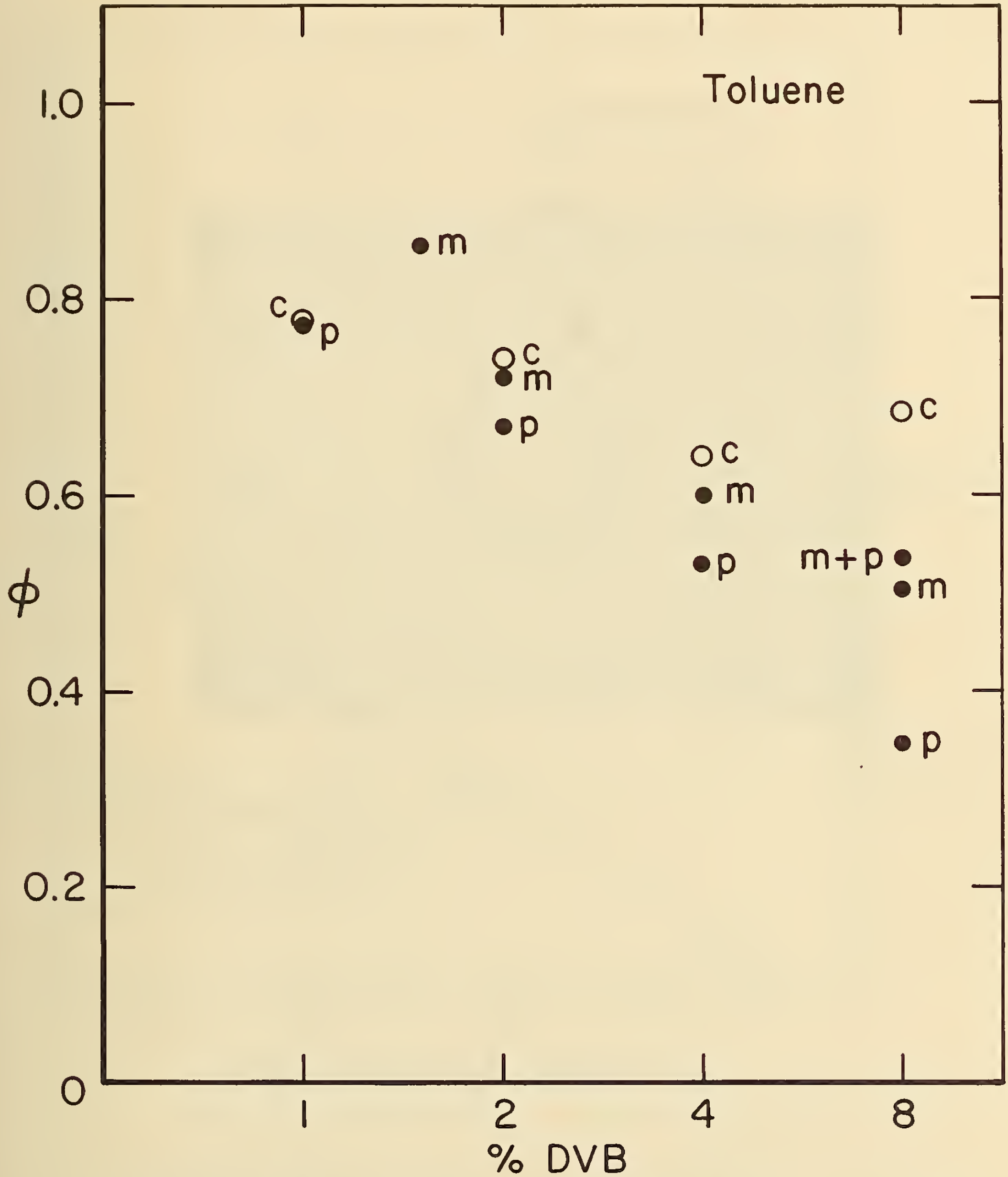
Commercial polymers quite often show large scatter among the individual beads (Figure 21) and poor correlation from



Infra-Red Absorption Ratios of Partially Sulfonated Styrene-DVB Copolymers (in KBr)

Figure 19. Capacity Relative to Adsorption for the 1408 cm^{-1} Band of Sulfonated Copolymers.

batch to batch. Transient deformations can occur at low DVB concentrations (eg. 2 percent) and are visible during the swelling process (Figure 22). More generally, a spherical shell structure is expected but it was not observed here.



Swelling Data in Toluene: Volume Fraction of Swelling $\phi = (q-1)/q$ versus Mole % DVB
p = para , m = meta, c = commercial

Figure 20. Mole Fraction of DVB Isomers Relative to Swelling Fraction ϕ [ϕ = (Q-1)/Q, where Q = swollen bead/dry bead diameter ratio].

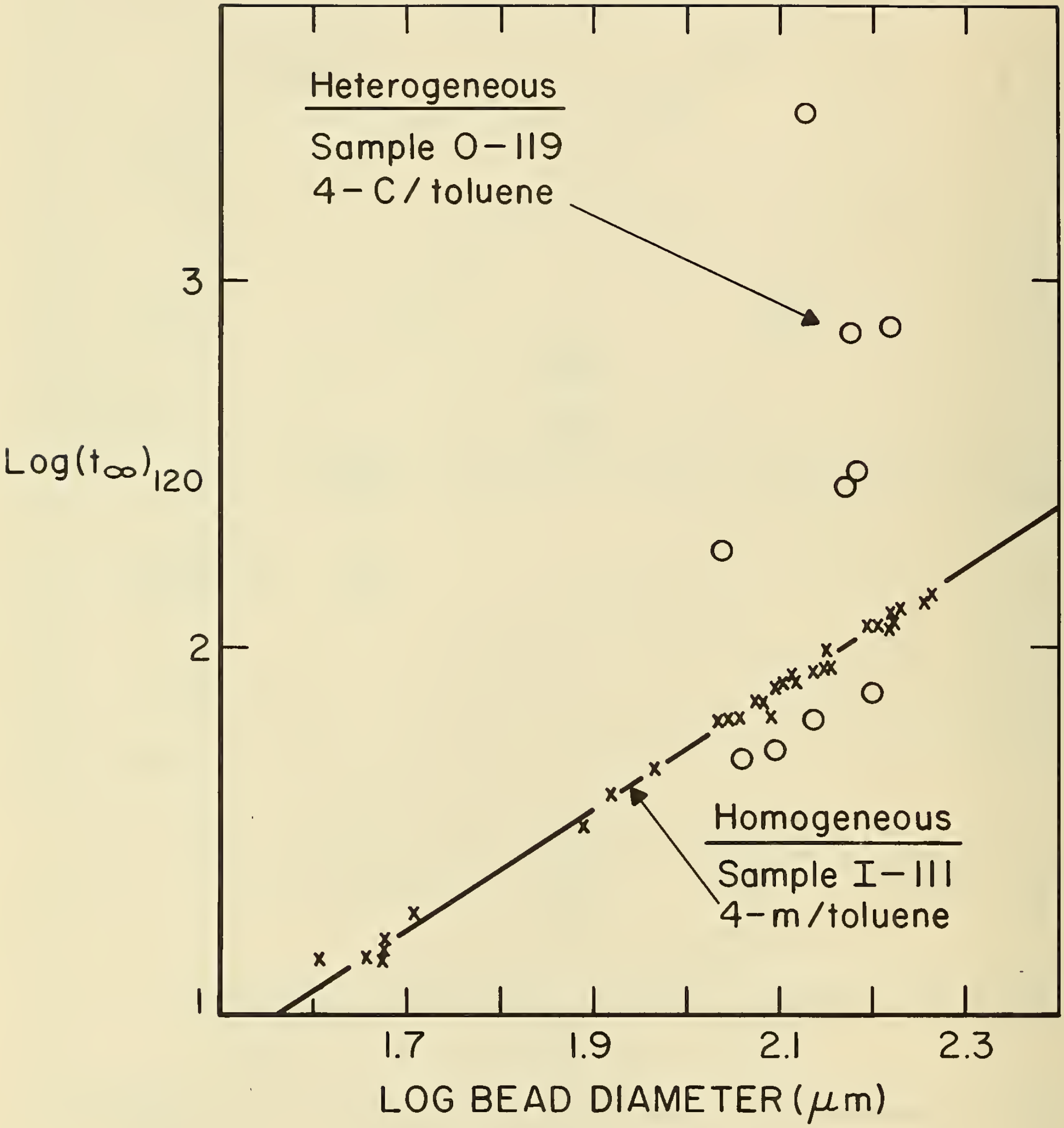


Figure 21. Log-Log Plot of Swelling Time Relative to Dry Bead Diameter Showing Homogeneous and Heterogeneous Swelling.

Figure 22. Photomicrograph Showing Heterogeneous Swelling.

III. COPOLYMER AND ION EXCHANGE MICROSCOPY

A. Introduction

With the advent of the use of ion exchange particles as carriers for chemical microstandards (see Section I), the technological problems of observance and manipulation of microscopic particles have demanded satisfactory solution.

It would be obvious that in order to satisfy such objectives, special equipment is required and special techniques must be employed if reliable Ion Exchange Microstandards are to be obtained.

This section describes some of the techniques and equipment which have been used in the microscopy laboratory to obtain satisfactory results with microstandard particles. No attempt however, is made here to explain treatment of the materials before they arrive or after they depart this laboratory.

B. Characterization of Copolymer Beads

One of the principal functions of the microscopy laboratory is the careful scrutinization of the various copolymer materials as they are received from various sources. Microscopic examinations are performed in order to determine their suitability and acceptance for further research or for use as ion exchange base material.

Photomicrographs of the materials are prepared using either normal transmitted or polarized light. A Control Number is assigned to each material, and all pertinent information and observations are entered into a Control Catalog for future reference.

The criteria normally used to reject materials from further study are (1) the entrappment of foreign particles in the network, (2) non spherical or broken particles, and (3) particles containing voids (holes) in the network. Once a material has been accepted for further study it is separated

into different size fractions by sieving. The size distribution of each sieve cut is determined by photomicrography. The micrographs are scanned with a comparator and measurements are made of the largest and smallest particles to give the size range of each cut (Figure 23).

Further characterization of the accepted material involves the swelling of particles in various organic solvents. Individual particles are selected and their diameters are measured in air. The particle is immersed in an organic solvent, the time for completed swelling is recorded, and the swollen diameter is then measured.

From such data, comparisons are made with other copolymers, crosslinking data are obtained, and decisions concerning ion exchange conversion are made.

Figure 24 shows a microscope equipped with an ocular micrometer, a counting cell, and a stop watch for swelling rate measurements.

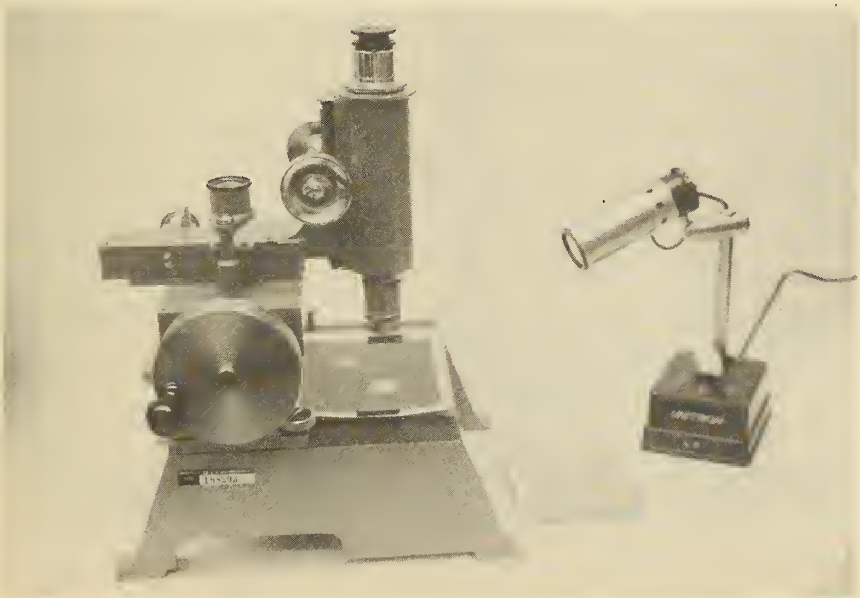


Figure 23. Comparator Used for Measurement of Particle Diameters and Particle Size Distribution.

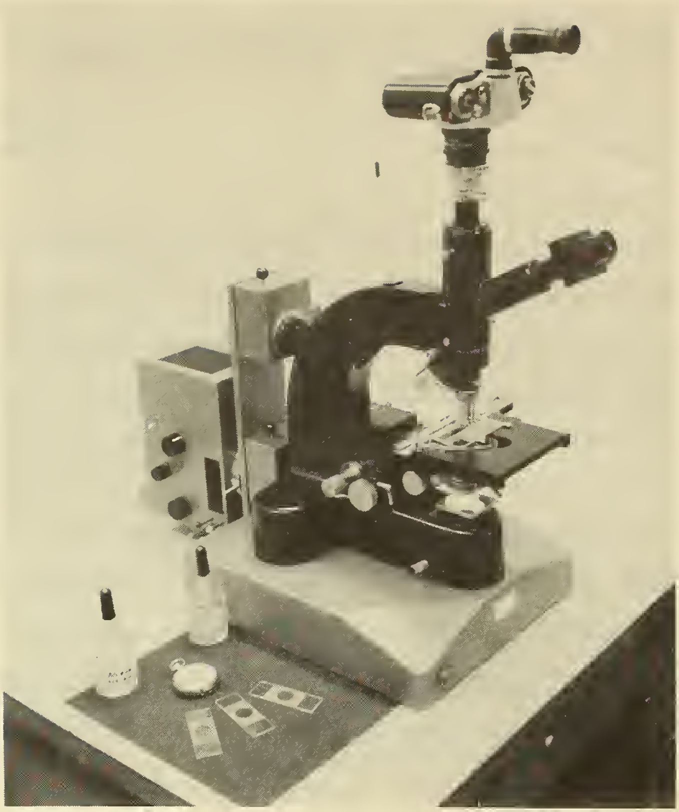


Figure 24. Microscope with Ocular Micrometer, Counting Cells, and Stop Watch for Swelling Studies.

C. Characterization of Ion Exchange Beads

In addition to copolymer samples, numerous commercial ion exchange resins are received by the microscopy laboratory. Each of these materials undergoes microscopic examination and cataloging in much the same manner as for copolymers. The material is then either accepted or rejected using the same criteria previously stated.

If a resin is accepted it must undergo chemical analysis, homogeneity testing, and density measurements to insure suitability for use as a chemical microstandard carrier. Ion exchange resins which meet specification for microstandard particles are subjected to electron microprobe analysis, mass spectrometry, activation analysis, and various chemical methods prior to certification.

Ion exchange particles examined by the electron microprobe are initially dispersed in a 0.1 percent solution of polyisobutylene in cyclohexane and cast on a polished aluminum or graphite disc. In the case of mass spectrometry, individual particles of measured diameter are selected and transferred directly to a filament which is then placed directly into the ion chamber of the spectrometer. For activation analysis, individually measured particles are selected following irradiation, and are mounted on a scintillation disc for counting.

Figure 25 shows a microscope with a micromanipulator and some of the various mounts used for the analysis of microstandard particles.

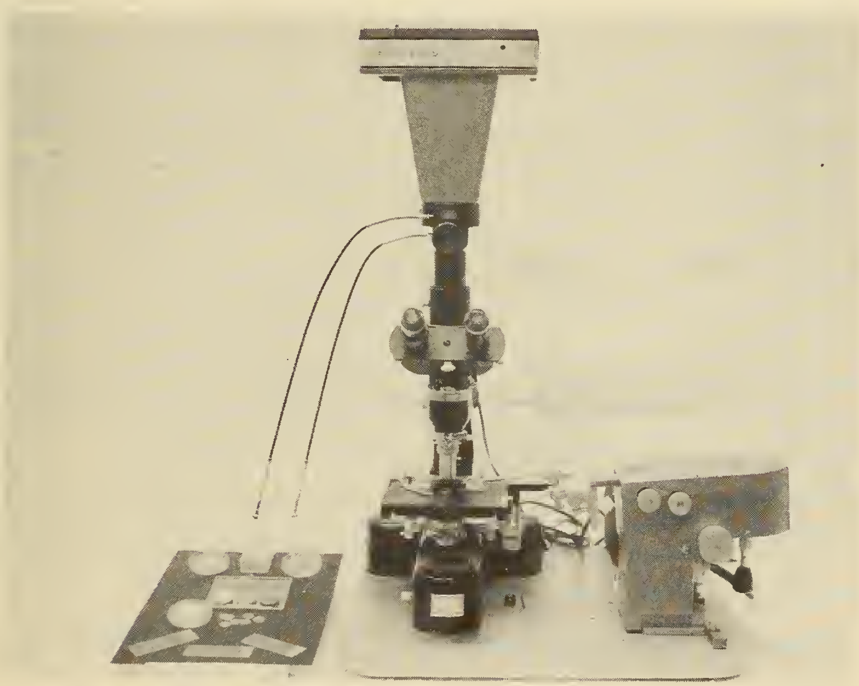


Figure 25. Microscope with Micromanipulator and Specimen Mounts for Microstandard Particles.

D. The Microscopy Laboratory Facility

The microscopy laboratory is equipped to perform conventional light microscopy via the use of transmitted light, incident light, phase contrast and fluorescence, with magnifications up to 1500 X.

In addition to the light microscopes an electron microscope is used to examine very small particles and to study structure defects and contamination in copolymer and ion exchange particles. The use of the electron microscope permits direct observations of these materials up to magnifications of 80,000 X.

Accessories to the electron microscope permit the stabilization of substances via a vacuum evaporator, the shadowing of specimens, and sample sectioning and preparation using a microtome. The photographic equipment employed for the preparation of photomicrographs obtainable from the laboratory's light microscopes consists of several polaroid cameras, a 4" x 5" plate camera, and a 35 mm camera. The electron microscope is equipped with both a 35 mm and a 2 1/4" x 2 1/4" plate camera. A photographic enlarger and a dry rapid print processor complement this equipment.

The present capability of the microscopy facility to observe microscopic defects, measure bead diameters with high precision, and perform transfers of loaded ion exchange particles, forms a necessary and unique function in the Ion Exchange Microstandards program.

IV. CHROMATOGRAPHY

A. Gas Chromatography

1. Divinylbenzene

Divinylbenzene (DVB) is widely used to prepare cross-linked poly(styrene/DVB) copolymers which may be derivatized (for instance, by sulfonation) to produce ion exchange resins. The importance of conducting analytical separations of DVB sample components is obvious in the potential extension of such studies to preparative scale separations to obtain the pure isomers of DVB for copolymer synthesis, as well as to provide detailed information regarding compositional makeup of commercial DVB.

(a) Separations on Bentone-34

Historically, most of the separations of DVB mixtures have been performed on a column composed of a mixture of 85:15:5 parts by weight of Chromosorb W: Bentone-34: Ucon oil [19], and examples of this are reported in detail in a previous NBS report [20]. Since this information was released, efforts were made to simulate detailed separations on Bentone-34 of the order reported by Hannah, *et al*. [16] on Carbowax 6000 and di-2-ethylhexyl sebacate. A chromatogram representative of our work is shown in Figure 26.

(b) Separations on Liquid Crystals

The term "liquid crystal" implies a misomer, but in reality such compounds evidence specific spatial structure in the liquid state. That is to say, a liquid crystal material does not become isotropic directly upon melting, but rather may exhibit molecular alignment in three principal stable structural phases prior to becoming isotropic; namely the smectic, nematic and cholesteric phase states. Liquid crystals as liquid phases in gas chromatographic separations provide an extra dimension of solute selectivity based upon the spatial geometry of their particular liquid state. Such materials

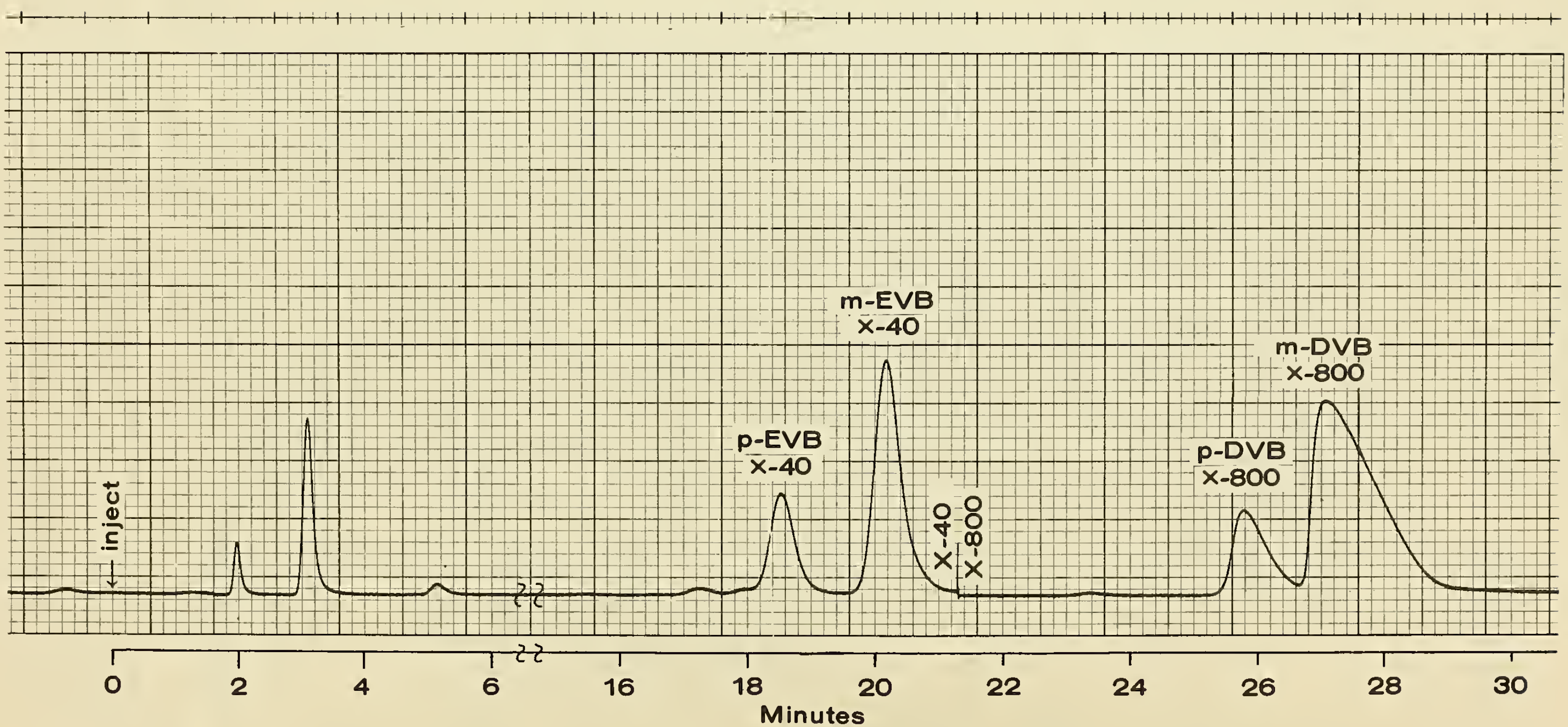


Figure 26. Separation of DVB Components on Bentone-34.

have been successfully employed recently for difficult isomeric aromatic separations by Kelker [21] in Germany and Martire [22] in the United States.

Separations of DVB components have been found to be quite dramatic on liquid crystals, specifically in the separation of the meta- and para-DVB, and meta- and para-ethyl vinylbenzene isomers (Figure 27). The liquid crystal employed for this work was recrystallized 4,4'-dihexoxyazoxybenzene (DHAB) at a 3 percent coating on 60/80 mesh acid-washed, DMCS-treated Chromosorb G in a 12 foot by 0.125 inch O.D. stainless steel

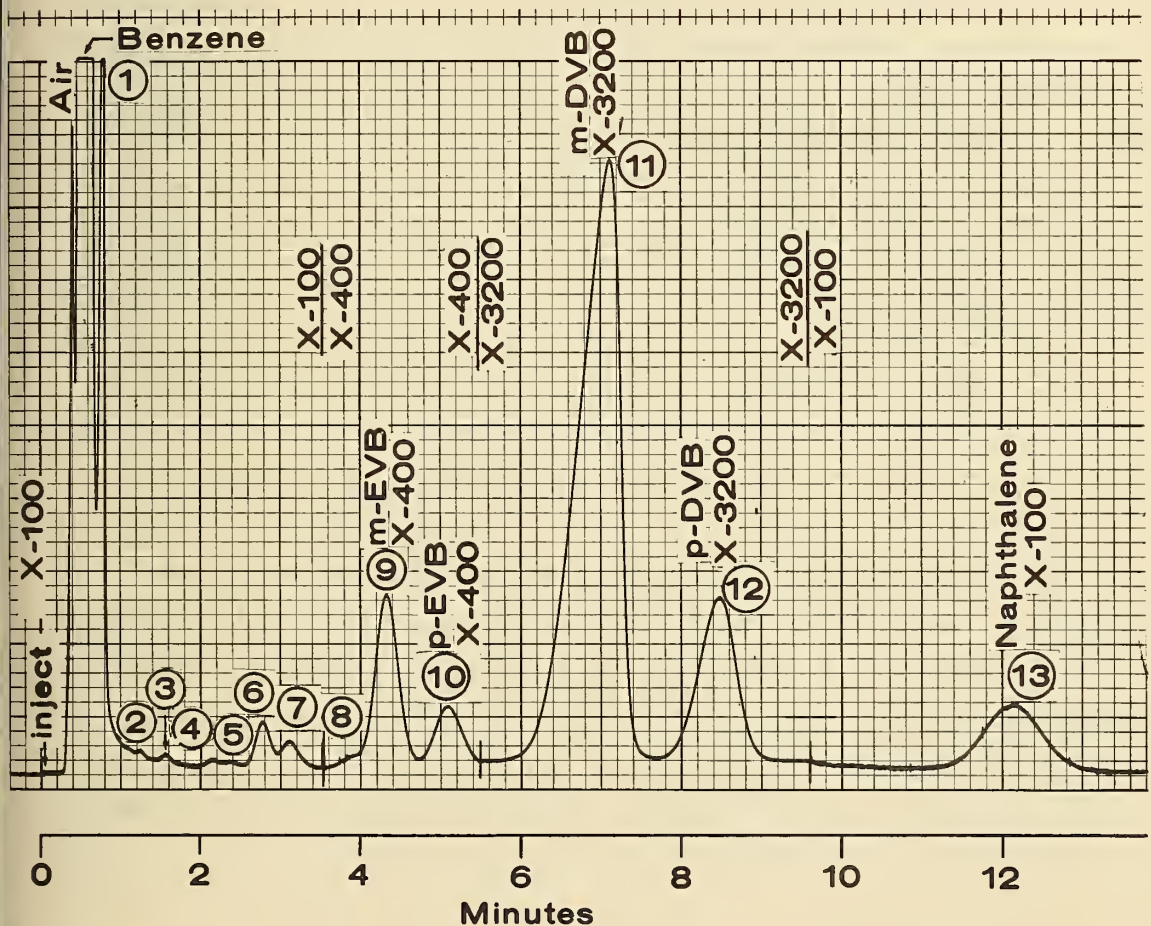


Figure 27. Separation of DVB Components on DHAB Liquid Crystal.

column. The analysis shown in Figure 27 was conducted at 127 °C. This liquid crystal melts at 81.0 °C into a nematic phase which becomes isotropic at 128.2 °C [23]. In the nematic phase state model, the liquid crystal molecules are arranged as non-layered parallel rods, enabling para-substituted aromatics to "fit" into and interact with the liquid crystal nematic phase more easily than meta and ortho substituted derivatives. Additional distinguishing features of analyses of DVB samples on liquid crystals relative to Bentone-34 columns provide (a) a much lower analytical temperature, (b) a faster analysis time, and (c) a realistic scale up of meta- and para-DVB separations to preparative scale gas chromatography. The α separation factor for meta- and para-DVB is better than 1.2 and a complete analysis can be conducted in less than 15 minutes. Complete preparative separation of all sample components is indicated by lowering the temperature further within the 81-128 °C nematic range. The data in Table III provides a comparison of analysis of a DVB sample on Bentone-34 vs. DHAB liquid crystal.

(c) Vinyl Toluene

Vinyl toluene (VT) has been examined as a candidate monomer in place of styrene for copolymer synthesis. Gas chromatographic analysis of commercial vinyl toluene, using a liquid crystal stationary phase, has revealed the presence of the para- (peak 5) as well as the meta-VT (peak 4) as major components, and six minor impurities (Figure 28). Peak area computations reveal that the meta (70.9 percent) and para (28.8 percent) isomers compose 99.7 percent of the sample. This information is essential for following reaction pathways using a VT monomer composed of the mixed isomers.

Table III. Gas Chromatographic Separation of DVB Components in Mixed DVB

Component	t'_R[a] A[d]	t'_R[a] B[e]	Relative Retention[b] A	Relative Retention[b] B	Relative Retention[b] C[f]	Percent[c] A	Percent[c] B
m-Ethylvinylbenzene	18.2	3.8	0.72	0.59	0.62	1.6	1.8
p-Ethylvinylbenzene	16.5	4.5	0.66	0.70	0.65	0.6	0.8
m-Divinylbenzene	25.1	6.4	1.00	1.00	1.00	77.7	79.0
p-Divinylbenzene	23.8	7.7	0.95	1.20	1.07	18.9	17.4
Naphthalene	35.3	11.2	1.41	1.75	2.07	0.4	0.4

[a] Adjusted retention time in minutes

[b] Relative to m-DVB as 1.00.

[c] Relative chromatogram peak area percentages

[d] Bentone-34 (NBS)

[e] DHAB liquid crystal (NBS)

[f] Carbowax 6000 (Ref. 16).

2. Other Chromatographic Studies

(a) Sulfolane

Sulfolane (tetrahydrothiophene-1,1-dioxide) was investigated by the Electrochemical Analysis Section as a potential solvent for EMF measurements. Despite its low molecular weight, it was not detected as a peak by gas chromatography. It was possible however to determine its water content at 0.6 percent by weight by the method of standard addition with an absolute error of less than 0.1 percent. An unidentified contaminant peak was also present at 3.7 times that of the water peak. Samples were dissolved in benzene and chromatographed on 80/100 mesh Poropak Q at 200 °C (Figure 29).

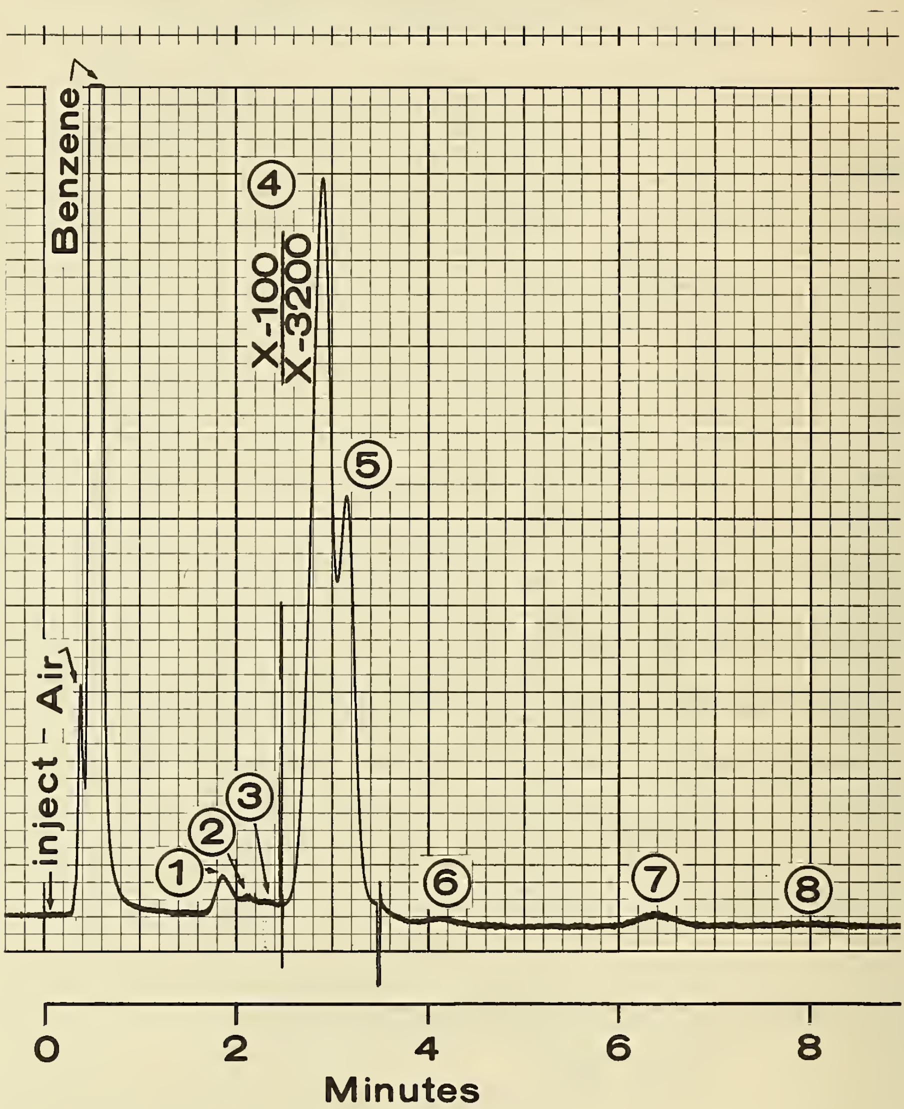


Figure 28. Analysis of Vinyl Toluene on DHAB Liquid Crystal.

(b) 2-Methoxy Ethanol

This material, commonly known as methyl cellosolve, has also been considered as a solvent for EMF measurements by the Electrochemcial Analysis Section. Commercial samples were evaluated by gas chromatographic analysis on 80/100 mesh Poropak Q at 200 °C using a thermal conductivity detector at a helium flow rate of 40 ml/min., for the presence of water and volatile impurities. The peak area response factors

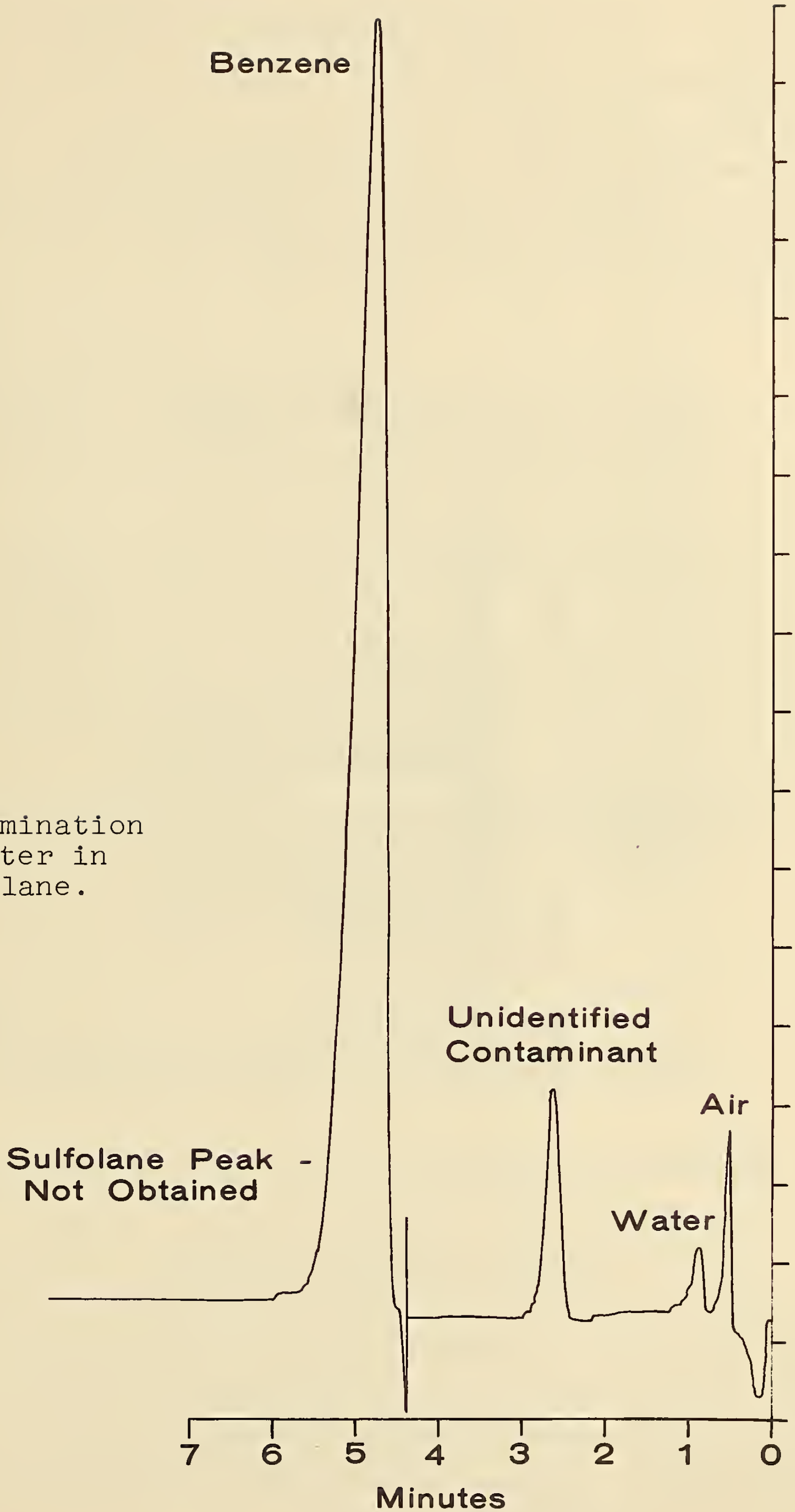


Fig. 29. Determination of Water in Sulfolane.

calculated separately for water and 2-methoxy ethanol were 1788 and 1080 mm^2/mg, respectively. Methanol, cited as a known degradation product of 2-methoxy ethanol, was not detected. The water content of the samples was found to be 0.036 weight percent. A chromatogram showing the separation of water, methanol, ethanol, and acetone on Poropak Q under these conditions is shown in Figure 30.

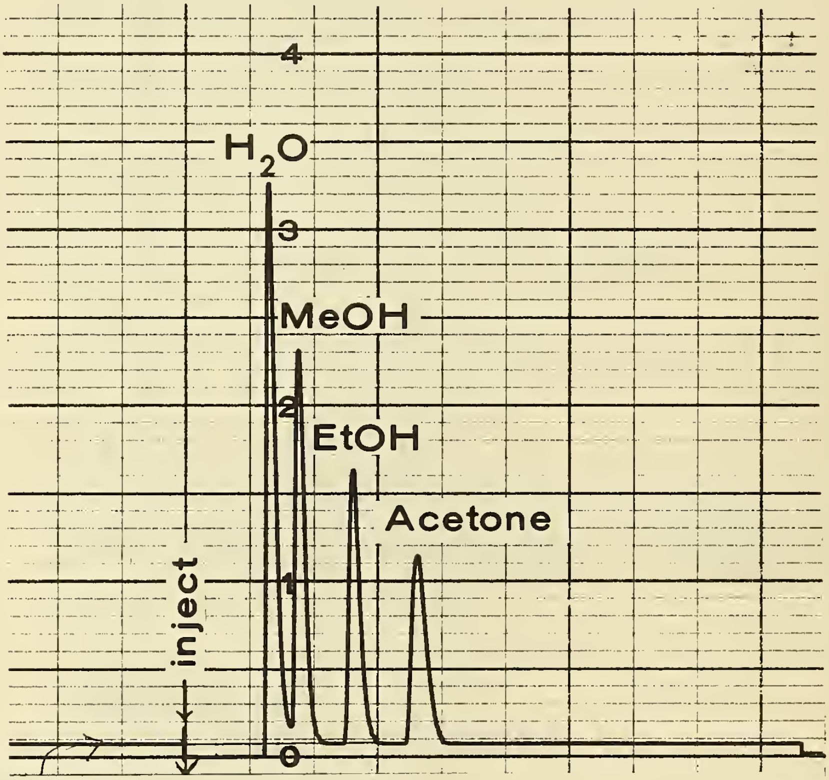


Figure 30. Separation of Volatiles on Poropak Q.

(c) <u>Ortho-Fluorobenzoic Acid</u>

This compound is issued as a Standard Reference Material (SRM 149) for microanalysis for fluorine. Gas chromatography of the silylated acid by temperature programming from 100 to 300 °C on 10 percent UC-W58 silicone coated on 80/100 mesh Chromosorb W using a flame ionization detector shows that analysis was simple and rapid.

3. Gas Chromatography-Mass Spectrometry

(a) The Quadrupole Mass Spectrometer

The quadrupole mass spectrometer is different from most other mass spectrometers because it utilizes only an electrostatic field rather than the magnetic and electrostatic fields of conventional magnet instruments to produce a mass spectrum. In conventional spectrometers the magnets required to produce an adequate magnetic field are necessarily large in contrast to the quadrupole system.

The sample enters the ionization region of the mass spectrometer as shown in Figure 31. The resultant sample

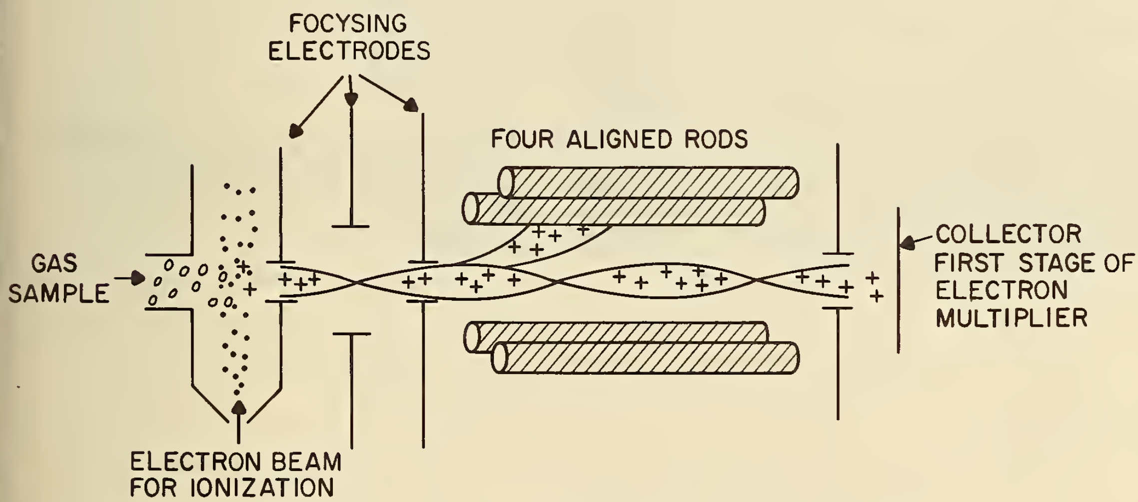


Figure 31. Quadrupole Filtering Schematic.

fragment ions are focused by the focusing electrodes and enter the quadrupole portion of the spectrometer. The quadrupole portion is composed of a mass filter which consists of two pairs of precisely aligned metal rods. The rods are electrically connected, in opposite pairs, to radio frequency (RF) and direct current (DC) potentials resulting in a hyperbolic electrostatic field. To produce a mass spectrum the RF and DC potentials are increased uniformly in time from zero to maximum potential. At a given time, ions of a

specific mass-to-charge (m/e) ratio are deflected as much toward one rod as they are toward another. Ions of this particular m/e value are initially excluded. As the voltages sweep from zero to their maximum values, the entire mass range (0 to 750 m/e) is scanned. The range thus starts at low m/e values and increases linearly to high m/e values.

Ions which avoid deflection and traverse the field are detected by an electron multiplier and produce an electrical signal which is amplified, and monitored on an oscilliscope. A recording of the mass spectrum can be initiated at any time by an oscillographic recorder contained within the unit to photograph the spectrum displayed on the oscilliscope. The system can produce a mass spectrum from m/e 10 to m/e 250 in less than three seconds with unit mass resolution.

(b) The GC-MS Interface

The ability of the quadrupole mass spectrometer to rapidly scan the m/e range of 0 to 750 renders it especially useful as a qualitative detector for organic vapors eluting from a gas chromatograph (Figure 32). The gas chromatograph however, operates at carrier gas pressures of a few atmospheres while the MS operates at pressures in the range of 10^{-5} to one atmosphere. In order to avoid swamping of the MS detector with ionized bulk carrier gas (He), an interfacing assembly is used to pump away most of the light GC carrier gas thereby enriching the gas stream entering the MS in sample eluent. The interfacing assembly is shown in Figure 33.

Gas flowing into portion A proceeds to the capillary portion D. In order to gain access to the MS, the gas must traverse the distance between capillaries D and C. The presence of a vacuum at this point removes most of the light helium carrier gas molecules from their D to C trajectory. Solutes of sufficient momentum (greater mass) eluting from the GC, proceed to point C and enter the mass spectrometer.

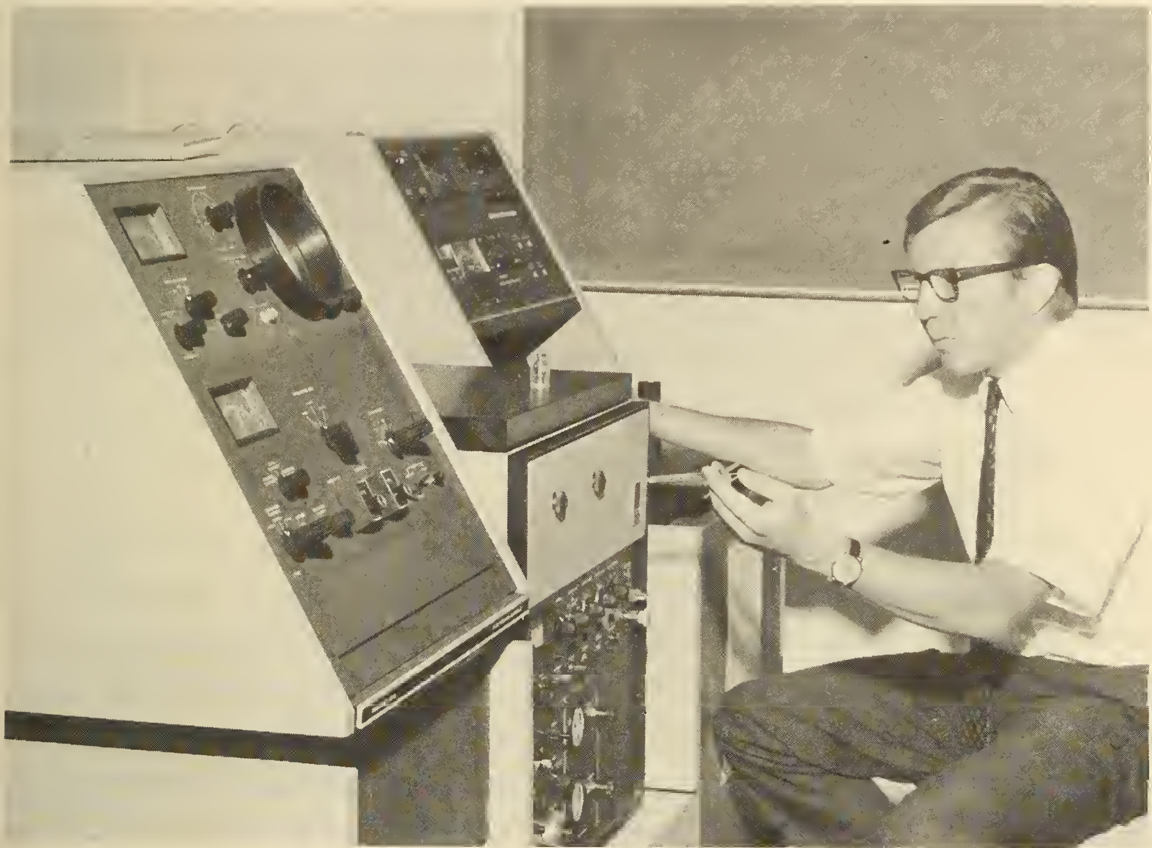


Figure 32. The GC-Mass Spectrometer System.

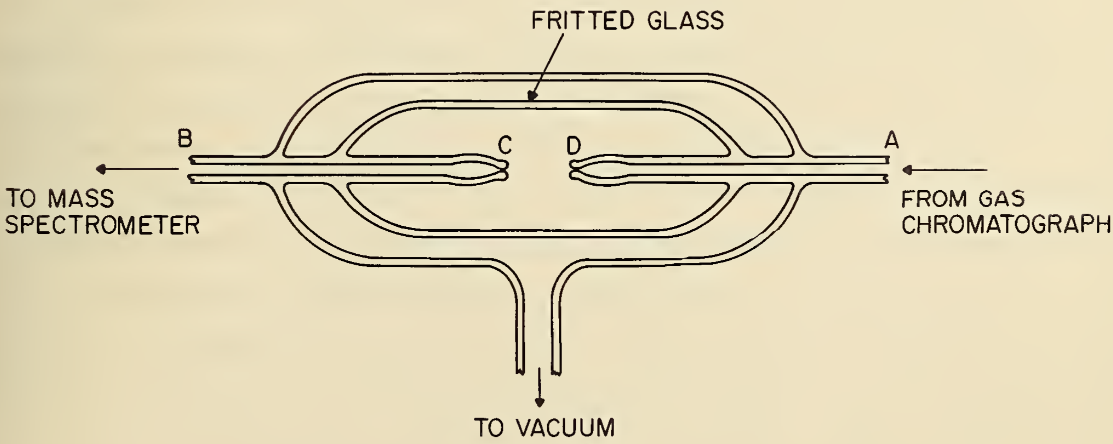


Figure 33. Schematic of GC-MS Interface.

The utility of combined gas chromatography-mass spectrometry will undeniably lend strong analytical support to the programs of the Separation and Purification Section in the areas of applied and basic research and development. This instrumentation is presently being applied to the identification of unknown components present in commercial divinylbenzene, and will be extended to the identification of isolated contaminants in clinical Standard Reference Materials.

B. Liquid Chromatography

1. Introduction

Recent developments leading to improved resolution and faster analysis in ion exchange and liquid-liquid chromatography have resulted in a resurgence of interest in these analytical techniques. Efficiencies and elution times in optimized systems can be made to approach those commonly experienced in gas chromatography. Column efficiencies in liquid systems may be expressed by theoretical equations analogous in form to the modified van Deemter equation for gas-liquid chromatography. An important exception is that the diffusion coefficient in liquids is four orders of magnitude smaller than in gases. The ability of a system to provide resolution is governed by the smallness and size uniformity of the particles employed as column packing material, the uniformity of the packed bed, the thinness of the film or ion exchange layer coating the particle, the smallness of sample sizes introduced into the column (which creates a requirement for sensitive detectors), and the narrowness of the column bore to minimize trans-column flow variations. All of these factors are pointed toward minimizing the theoretical plate height for a given column length and solvent carrier flow rate. The use of very small particles (10-50 microns in diameter) produces a sizeable resistance to flow such that, to insure that retention times of solutes are not prohibitive, pressures on the order of 100-1000 p.s.i. are

used with thick-walled glass columns (for example: 0.25" I.D. with a 0.125" wall). Pressures as great as 3000 p.s.i. or higher require the use of stainless steel columns, where column bore diameters as small as 2 mm are appropriate.

2. Instrument Design

A liquid chromatograph suitable for liquid-liquid, liquid-solid, and ion exchange is presently in use in our laboratories. High inlet pressures require reliable pumps. A precolumn or a presaturated carrier solvent reservoir is used with liquid-liquid chromatography to presaturate the mobile solvent phase, which should be adequately degassed to avoid the escape of air bubbles at the low pressure end of the column or in the detector. Pressures and liquid flow rates are carefully monitored, and the feature of measured column thermostatting is desirable. The injection port should not allow the entrance of air upon sample injection, and a high-quality high-pressure valve with a changeable sample loop (as used by Scott at ORNL) has been concluded to be nearly ideal. However, septum injection utilizing a double septum in the injection port has also been found to work well at inlet pressures up to 800 p.s.i. (Figure 34). The connection from the column exit to the detector flow cell inlet (Figure 35) and the flow cell itself should be as small in volume as possible. Finally, the detector should be highly sensitive to compositional changes in the column effluent, having a low electronic noise level, fast response, and preferably low selectivity to solute type. Although no specific detectors are suggested, the differential refractive index detector and the ultraviolet photometric detector (Laboratory Data Control, Danbury, Connecticut), and the microadsorption detector (under investigation at Oak Ridge National Laboratories), are detection devices which we have considered. Acknowledgment for considerable assistance obtained in suggestions and informative discussions are owed

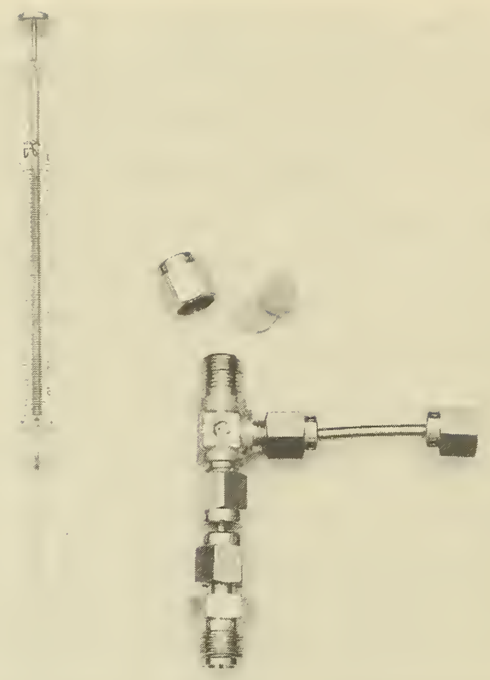


Figure 34. Sample Inlet System for Septum Injection.

to Dr. Paul B. Hamilton (Alfred I. du Pont Institute, Wilmington, Delaware), Dr. Charles Scott (Oak Ridge National Laboratories, Oak Ridge, Tennessee) and Dr. Jack Kirkland (E. I. du Pont de Nemours, Wilmington, Delaware), among many others.

3. Clinical Standard Reference Materials - Bilirubin

Liquid chromatography permits the analysis of underivatized high molecular weight, heat-labile compounds which may not be successfully analyzed by gas chromatography. Numerous and diverse clinical materials of a biological nature tend to fall into this domain. The necessity for accurate separation and measurement of impurities in such compounds forms a part of our clinical Standard Reference Materials program. The Analytical Chemistry Division is involved in the examination of bilirubin samples obtained from various sources. The bilirubin molecule contains two vinyl, two propionic acid, four methyl, and two tautomeric hydroxyl residues, attached

Figure 35. Connection from Column Exit to Detector.

to four pyrrole rings and is clinically used as a diagnostic indicator for jaundice. Bilirubin is unstable when under exposure to light and heat, in certain basic solutions, and is oxidizable under mild conditions to biliverdin. The chromatography of this compound has been obtained on a preliminary system at an inlet pressure of 570 p.s.i.g. on an aluminum foil-covered column packed with a 10-30 μm cation exchange resin using a differential refractive index detector (Figure 36). A schematic of this system is presented in Figure 37. The analysis of an impure bilirubin sample obtained on this system is shown in Figure 38. The carrier solvent used in this chromatogram had the composition 0.02 M NH_4OH-0.02 M NH_4Cl. The stability of bilirubin in this solvent is not

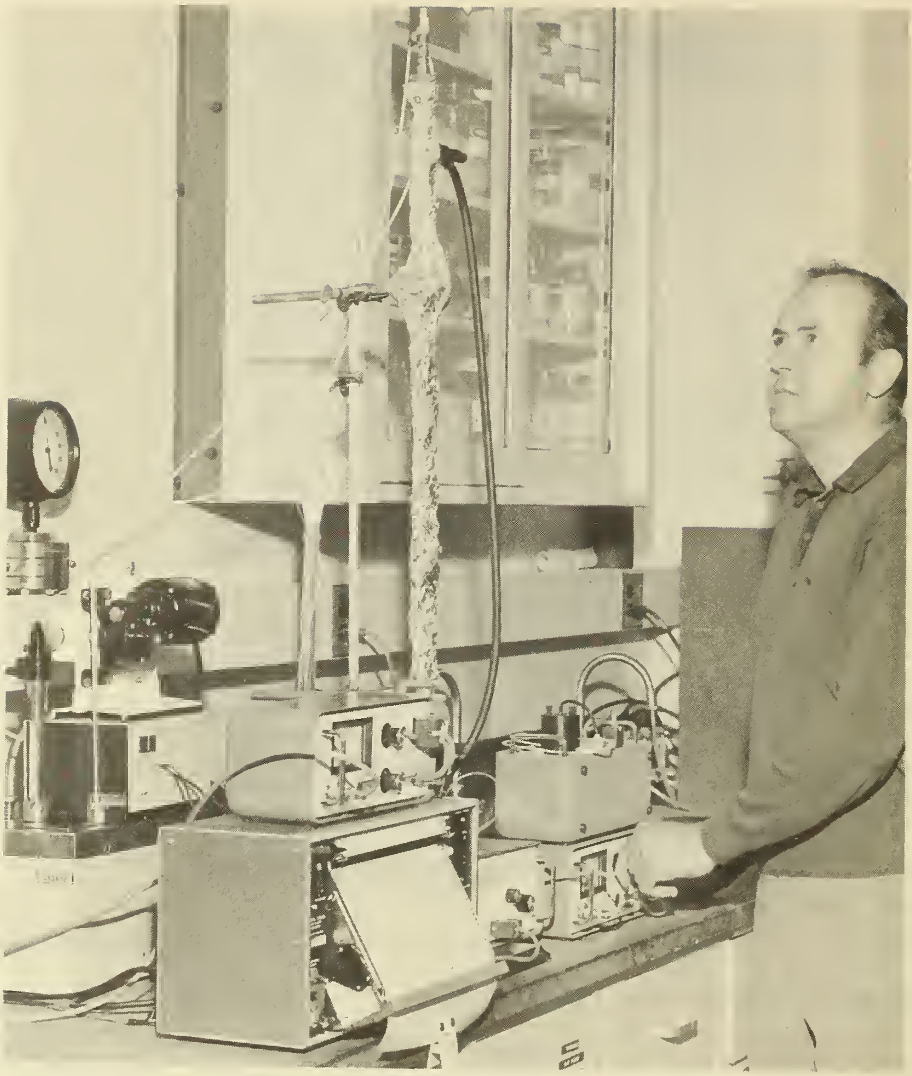
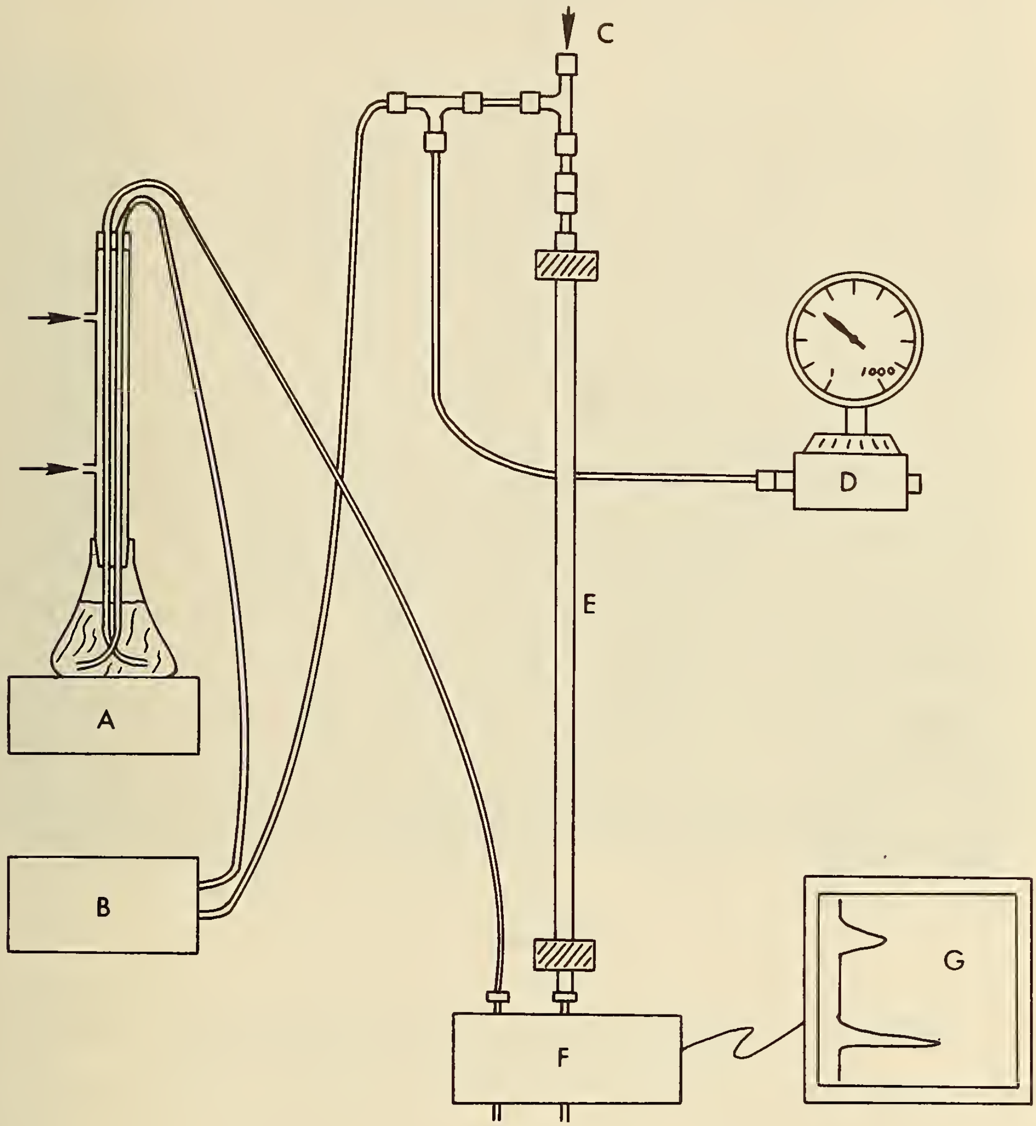


Figure 36. Preliminary Ion Exchange Chromatographic System.

ideal. Other solvents for the chromatography of bilirubin are being considered. Its chromatography in dimethylsulfoxide (DMSO), an excellent solvent for bilirubin, is under investigation. Spectrophotometric measurements in DMSO gave a comparable final reading (0.795 A) to the initial reading (0.794 A) over a period of forty minutes. The molar extinction coefficient for bilirubin in DMSO was calculated from this data to be 61.92×10^3.



A. Hot plate supporting solvent reservoir
B. Pump
C. Sample inlet (syringe injection)
D. Pressure gauge
E. Analytical column
F. Detector (R.I. or U.V.)
G. Recorder

Figure 37. Schematic of Ion Exchange Chromatographic System.

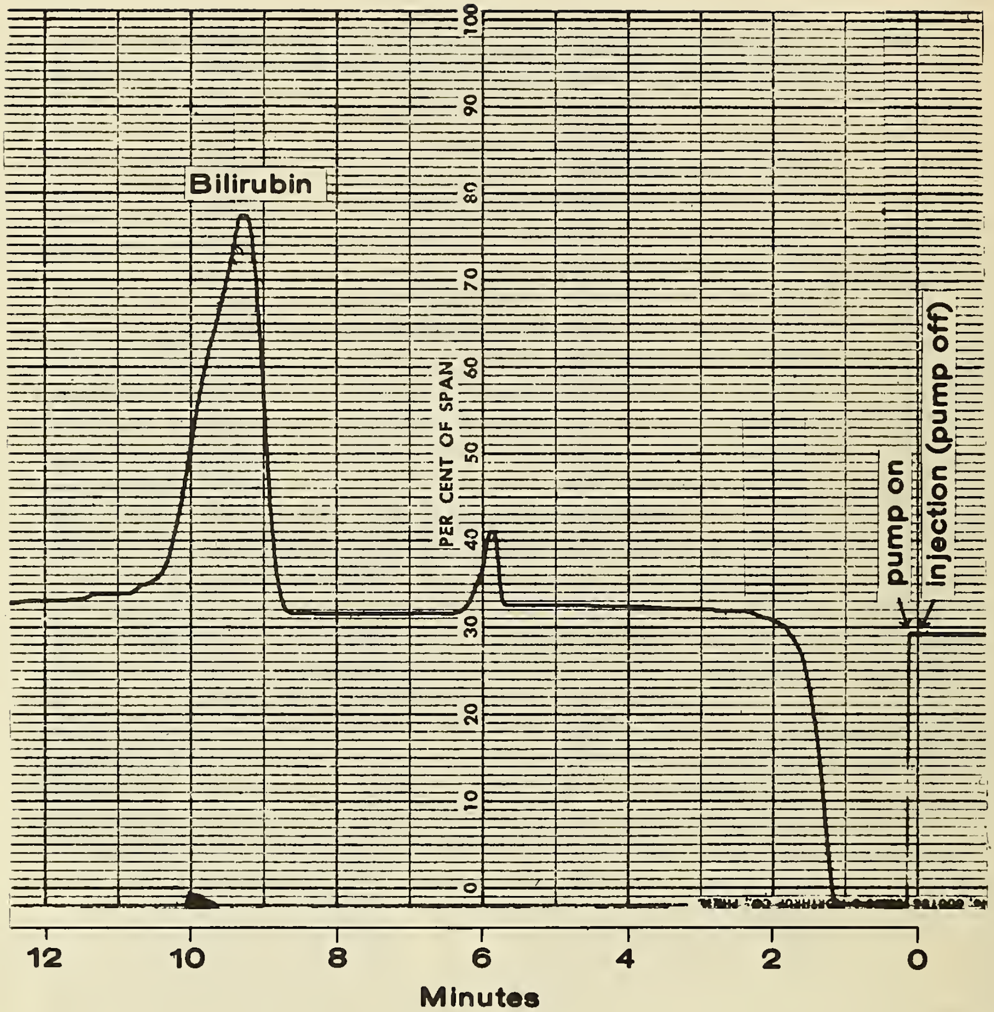


Figure 38. Analysis of an Impure Bilirubin Sample by Ion Exchange Chromatography.

V. REAGENTS

A. Introduction

The increasing sensitivity of instrumental analysis, coupled with the rising significance of trace impurities in wet chemical analysis, necessitates the availability of reagents whose purity criteria far exceed those of the normal "CP" or "reagent grade" classification. This is especially true at the National Bureau of Standards where reagents with impurities measurable in parts per billion must now be available. When reagents in this purity range are needed in the Analytical Chemistry Division it is the obligation of this Section to make them available.

While formerly it was necessary to synthesize such materials ourselves, the potential market in ultra pure chemicals has encouraged commercial firms to expand in a direction to fulfill this need. The more common chemicals can be purchased in quantity, while more specialized ones can often be obtained by specific order. These aspects of our reagent program are covered in Section B, below. There are, however, instances when commercial sources cannot answer our problems, and the synthetic work must be done by this Section, as is discussed in Section C, below.

In addition to fulfilling the immediate needs for ultra purity in the reagent field, research on certain aspects of contamination removal has been undertaken. The use of a microturbidimeter to accurately measure particulate matter to one part per billion in high purity reagents is shown in Figure 39. Previous studies on reagent contamination via entrained foreign materials available in container walls have been continued and are reported in Section D below.

Figure 39. Microturbidimetric Measurement of Particulate Matter Content.

B. Ultra Pure Reagents Services

In recent years it has been possible to purchase from commercial sources the more common reagents in ultra pure form and to maintain a stock of these chemicals on hand. To retard their decomposition during storage, the chemicals are kept in freezers. A selection of such chemicals available for immediate "self-service" distribution is kept at 0 °C, while a larger quantity is kept on reserve at -30 °C. Of the 27 separate reagents stocked for direct use, 145 items were dispensed over the past year (Table IV). This is an increase of roughly 250 percent over the previous year.

Table VI. Number of Units of Ultra Pure Reagents Dispensed in FY 1969.

Acids		Bases	
CH_3COOH	2	NH_4OH	21
HBr	2	NaOH	1
HCl	19		
HF	8	**Redox**	
HI	0	Br_2	1
HNO_3	24	I_2	0
$HClO_4$	19	LiF	1
H_2SO_4	9	KI	2
		$Na_2S_2O_3$	1
Salts		CsI	3
$CaCl_2$	4	NaI	1
LiBr	2		
KCl	3	**Buffer Salts**	
NaCl	5	NH_4Cl	3
$BaCl_2$	2	CH_3COONa	3
BaF	1	Na_2SO_4	1
$Mg(NO_3)_2$	2		
NaF	3		
Na_2CO_3	2		

In addition, special requests for specific reagents needed by people in this or other Sections at NBS have been met through contact maintained by us with commercial manufacturers. For example, the difficulty in obtaining lead-free phosphoric acid for mass spectroscopy was solved by advising its preparation from re-sublimed phosphorous pentoxide. A sample of ULTREX P_2O_5 (J. T. Baker Co., Phillipsburg, New Jersey) was used in this instance.

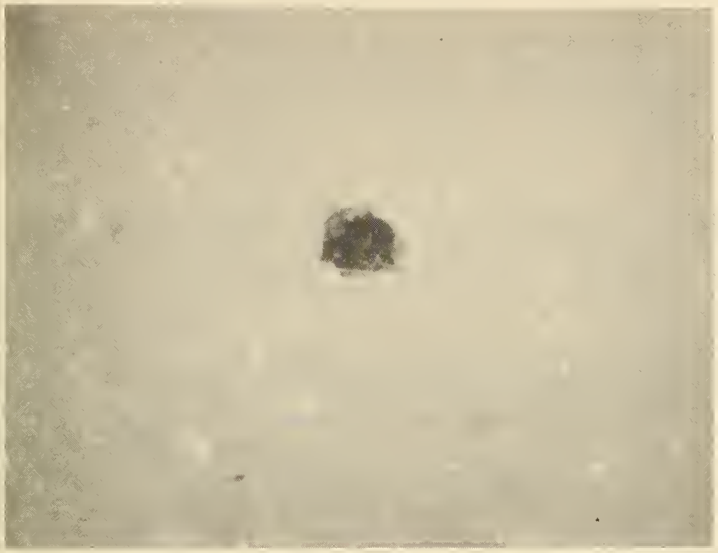
C. Ultra Pure Reagent Synthesis

For the Section's Microstandard program a need arose for ultra pure inorganic chemicals which could be loaded on ion exchange beads. Spectrographically standardized reagents (Johnson, Matthey Co., Ltd., England) are used for this purpose. However, it is not always possible to purchase directly the chemical compound desired; the requisite compound must then be synthesized from some other available ultra pure compound. In preparing the ion exchange microstandards of chromium and magnesium only the insoluble oxides were obtainable. Therefore, the suitable chemical forms, chromium (III) perchlorate and magnesium chloride were prepared from the respective oxides.

D. Container Purification

In a previous report [1] it was noted that the constant iron contamination of re-distilled hydrochloric acid was traceable to iron-containing particles embedded in the polyethylene receiver. The removal of such entrained container contamination became a matter of necessary concern.

The same problem was encountered using Teflon FEP bottles. Here however, the inertness of the container material permitted drastic chemical methods for contamination removal from the container, even for contamination existing beneath the container surfaces. Figure 40 illustrates the result of using boiling nitric acid in removing such entrainments.



Photomicrograph of opaque particle embedded in wafer of Teflon FEP. Particle is positioned ca. 70 microns below surface. (125X)

Photomicrograph as per above, but after 60 hours of immersion in boiling nitric acid. (125X)

Figure 40. Effect of Boiling Nitric Acid in Removal of Container Contaminants.

VI. PERSONNEL AND ACTIVITIES

A. Personnel Listing

Separation and Purification Section

David H. Freeman, Section Chief
Janice M. Hurst, Secretary

Ion Exchange

Rosalie Angeles (Part time)
Lawrence Morgenthaler (Summer faculty participant from Georgetown University)
Robert Young (Summer student participant from Georgetown University)

Chromatographic Separations

William Dorko
Delmo Enagonio
Walter Zielinski
Sister Laura Turbini (National Science Foundation Summer Institute for Teachers of Physics and Chemistry)

Particulate and Microscopic Studies

Patti Byrne (Guest Student Worker, University of Delaware)
Jane Doyle

High Purity Reagents

Edwin Kuehner
Gerald Sleater

Microscopic Standards

Herbert Dixon
Vincent Story

Youth Opportunity Corps

Linda Gant
Sylvia Prather

B. Talks

1. D. H. Freeman, W. F. Rittner, "Studies of Ion Exchange Resin Structure," 156th National Meeting of the American Chemical Society, Atlantic City, New Jersey. September 11, 1968.

2. D. H. Freeman, "Particles in Solution by Laser-Light Scattering," Scientific Apparatus Makers Association, National Bureau of Standards. September 17, 1968.

3. D. H. Freeman, "Microstandards for Activation Analysis," 1968 International Conference on Modern Trends in Activation Analysis, National Bureau of Standards. October 8, 1968.

4. D. H. Freeman, "Ion Exchange Resin Standards: Characterization and Applications," Howard University, Washington, D. C. November 21, 1968.

5. D. H. Freeman, "Chemical Microstandards," American Microchemical Society, Hoboken, New Jersey. December 12, 1968.

6. D. H. Freeman, "Chemical Microstandards," American Chemcial Society, 4th Annual Middle Atlantic Regional Meeting, Washington, D. C. February 13, 1969.

7. D. H. Freeman, "Chemical Microstandards," 20th Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Cleveland, Ohio. March 5, 1969.

8. D. H. Freeman, "Studies of Ion Exchange Networks," Georgetown University, Washington, D. C. March 19, 1969.

9. W. L. Zielinski, Jr., "High-Resolution Liquid Chromatography," Washington Gas Chromatography Discussion Group, Rockville, Maryland. May 15, 1969.

10. D. H. Freeman, "Light Scattering Measurements of Sub-PPM Concentrations of Liquid-borne Particulate Matter," 1969 ASTM Convention, Atlantic City, New Jersey. June 23, 1969.

C. Publications

1. D. H. Freeman, "Separation and Purification Section: Summary of Activities July 1967 to June 1968," NBS Technical Note 459, (1968).

2. D. H. Freeman, Book Review: "AnalaR Standards for Laboratory Chemicals," Anal. Chem. 40, 75A (1968).

3. D. H. Freeman and E. C. Kuehner, "Containers for Pure Substances," (Chapter 30) in Purification of Inorganic and Organic Materials, Marcel Dekker, Inc., New York, N. Y., 299-306 (1968).

4. D. H. Freeman, W. L. Zielinski, Jr., and W. F. Rittner, "Recognition of Crosslinking in the Infrared Spectra of Poly(styrene/divinylbenzene)," accepted for publication in the Proceedings of the International Conference on Ion Exchange in the Process Industries.

5. D. H. Freeman, Book Review: "Reagent Chemicals," Anal. Chem., 41, 87A (1969).

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